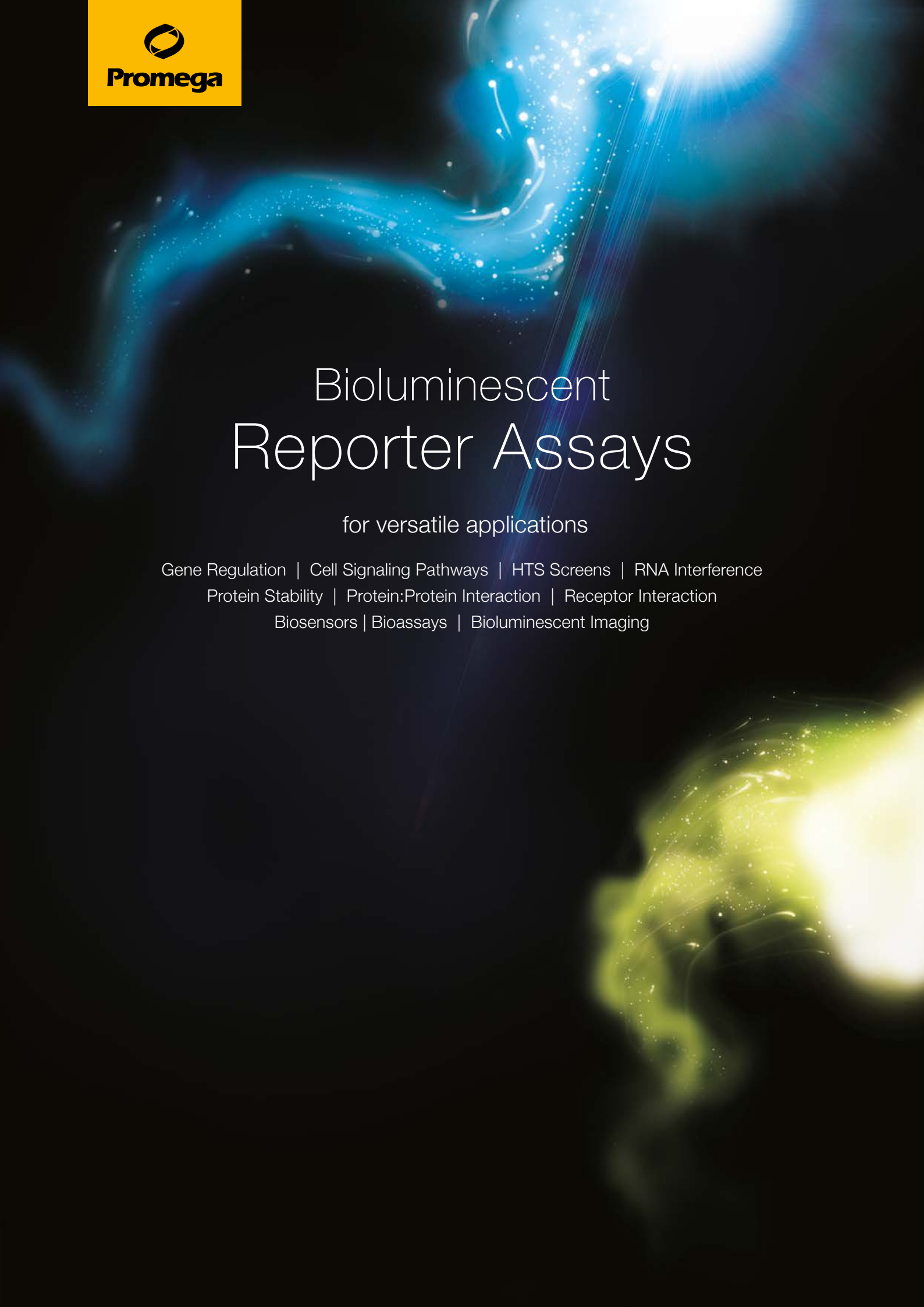

Promega
Bioluminescent
Reporter Assays
for versatile applications
Gene Regulation | Cell Signaling Pathways | HTS Screens | RNA Interference
Protein Stability | Protein:Protein Interaction | Receptor Interaction
Biosensors | Bioassays | Bioluminescent Imaging

The Brightest Luciferase Reporter

Smartphone image of a 384-well plate
with NanoLuc®-expressing cells.

March 2020_REG4040

Supporting Science Around the World

With a portfolio of more than 3,000 products covering the fields of genomics, protein analysis/expression, cellular analysis, drug discovery, and genetic identity, Promega is a global leader in offering innovative solutions and technical support to scientists in academic, industrial and governmental settings. Promega provides products globally through its 15 branches and over 50 distributors. Serving more than 100 countries, Promega supports laboratories with its molecular tools, technical support, and customer service. Promega was first certified to ISO standards in 1998. Promega Madison, USA, maintains its ISO 9001 and ISO 13485 certification for the manufacture of medical devices. Currently, 15 Promega facilities around the world are ISO-certified.

One of Promega's core competencies is the continuous development of innovative luciferase-based technologies. Within this brochure, discover novel and approved technologies to explore the exciting field of cellular biology!

1 Introduction

1.1 Luciferase Reporter Assays

Luciferases are widely used as reporter genes across various fields of cell biology, including the analysis of gene expression at the transcriptional, translational, and post-translational level, the generation of biosensors and the detection of protein interactions in live cells. The term luciferase refers to a class of enzymes catalyzing a light-emitting reaction by oxidation of their respective substrate. This so-called “bioluminescence reaction” enables easy quantification of luciferase reporter activity with an exceptionally high sensitivity and a wide dynamic range. In comparison to fluorescent reporter assays, the sensitivity of luciferase-based assays is about 10- to 100-fold greater and the linear range spans up to eight orders of magnitude. The background noise of luciferase reporter assays is negligible since light develops where darkness once prevailed. Luciferase reporter assays exhibit a robust assay chemistry with a considerably low substance interference and thus are widely applicable from single measurements to high-throughput screenings (HTS).

Applications of Luciferase Reporter Assays

- Promoter studies
- Gene regulation
- Cell signaling pathways
- Compound screening
- Bioassays
- Protein:protein interactions
- Protein:ligand interactions
- Protein degradation
- Post-translational modifications
- Virus-cell interactions

1.2 NanoLuc® – Renilla – Firefly: A Comparison of Luciferases

Luciferases are well suited for setting up highly sensitive and easy-to-quantify reporter assays. Traditionally, a combination of firefly and Renilla luciferase is applied to study gene regulation. The firefly luciferase (Fluc) originates from the American beetle *Photinus pyralis*, while Renilla luciferase (Rluc) derives from the sea pansy *Renilla reniformis*. By contrast, the newly developed and highly optimized NanoLuc® luciferase (Nluc) originates from the deep-sea shrimp *Oplophorus gracilirostris*. Its markedly increased signal intensity as well as its considerably small molecular size display key advantages over other commonly applied luciferases. Therefore, Nluc is ideally suited for challenging applications, i.e. reporter studies at physiologically relevant expression levels or protein interaction studies in live cells.

We will gladly assist you in selecting the optimal reporter system tailored to your needs!

A comparison of luciferases: Firefly – Renilla – NanoLuc®

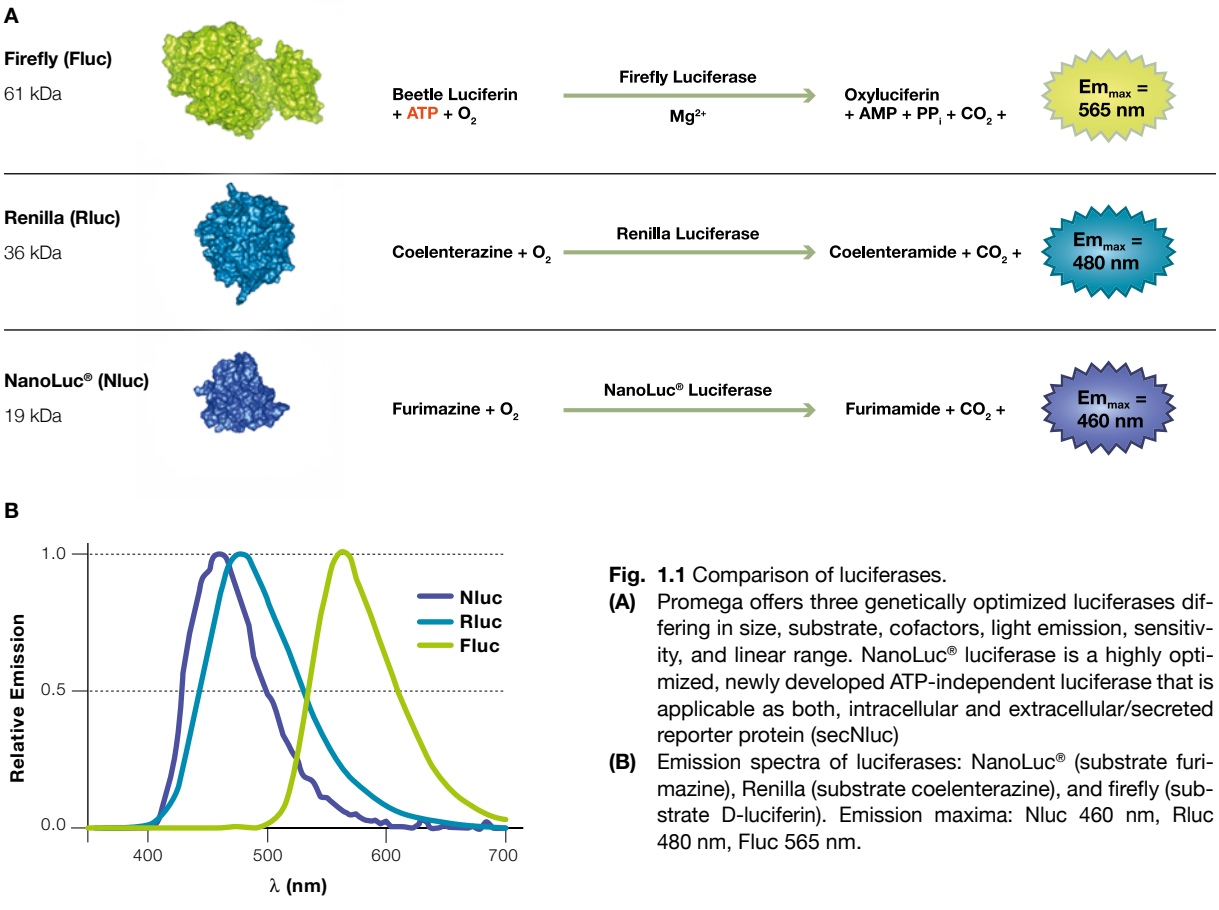


Fig. 1.1 Comparison of luciferases.
(A) Promega offers three genetically optimized luciferases differing in size, substrate, cofactors, light emission, sensitivity, and linear range. NanoLuc® luciferase is a highly optimized, newly developed ATP-independent luciferase that is applicable as both, intracellular and extracellular/secreted reporter protein (secNluc)
(B) Emission spectra of luciferases: NanoLuc® (substrate furimazine), Renilla (substrate coelenterazine), and firefly (substrate D-luciferin). Emission maxima: Nluc 460 nm, Rluc 480 nm, Fluc 565 nm.

1.3 NanoLuc® – The Brightest Luciferase Reporter

NanoLuc® luciferase (Nluc) is a genetically optimized luciferase that has been developed by directed enzyme evolution of a luciferase from the deep-sea shrimp *Oplophorus gracilirostris*. Nluc catalyzes an ATP-independent conversion of its optimized substrate, furimazine, yielding luminescence 150-times brighter than FLuc or RLuc (Fig. 1.2). Furthermore, the Nluc substrate furimazine displays enhanced stability with a half-life greater than two hours and a lower background activity, which both open up a new field for bioluminescence imaging. With a molecular weight of only 19 kDa, Nluc is substantially smaller than firefly and Renilla luciferases. This offers several advantages for various applications such as the set up of viral infection assays or protein interaction assays. Nluc exhibits a high physical stability and retains its activity at temperatures of up to 55°C or in cell culture medium at 37°C (>15 hours). Thus, this enzyme provides all fundamental features to set up sensitive and robust reporter assays.

NanoLuc® luciferase has been successfully tested in the following applications:

Reporter gene assays (Kobayashi, EH *et al.* (2016) Nat. Comm. 7, 11624)
Protein stability (Robers, M (2014) PubHub 2/2014; tpub 139)
Protein interaction (Machleidt, T *et al.* (2015). ACS Chem Biol. 10:1797-804)
Protein ligand binding (Stoddart, LA *et al.* (2015) Nature Methods. 2:661-3)
Target engagement (Robers, MB *et al.* (2015) Nat Commun. 6:10091)
Target identification (Ohana, RF *et al.* (2015) ACS Chem Biol. 10:2316-24)
Receptor endocytosis (Robers, MB *et al.* (2015) Anal Biochem. 489:1-8)
Bioluminescent imaging (England, CG *et al.* (2016) Bioconjug Chem. 18; 27:1175-87)
Monitoring of virus replication (Sun, C *et al.* (2014) J Virol. 88:2035-46)

Advantages of Nluc's high signal strength

- High sensitivity, robust signals
- Higher signal strength featuring low-level reporter expression
- Applicable to hard-to-transfect cell lines
- Suitable for *single copy* applications
- Improved bioluminescent imaging

Comparison of luciferase activities

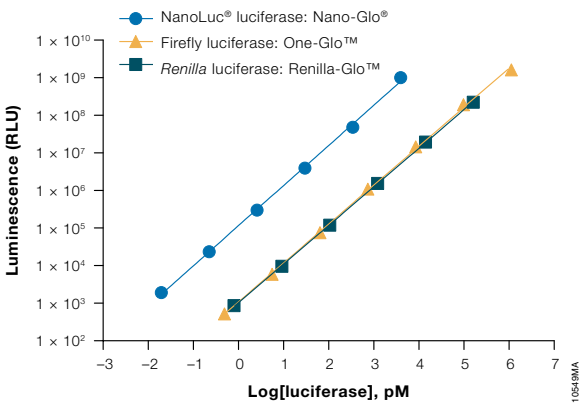


Fig. 1.2: NanoLuc® luciferase shows a 150-fold greater specific activity in comparison to firefly and Renilla luciferases.

1.4 Experimental Design of Reporter Assays

The experimental design is critical to the success of any reporter assay. Generally, it can be divided into the following key steps: ① cloning of reporter vector constructs, ② transfection & cultivation of cells, and ③ signal development & detection (Fig. 1.3). Usually, the most time consuming step is the generation of a reporter construct whose respective design depends on the particular scientific question (Fig. 1.4). Studies on transcriptional regulation – e.g. promoter studies – require the insertion of a regulatory DNA sequence into a promoterless vector upstream of the luciferase gene. Alternatively, the luciferase-encoding DNA sequence is directly integrated into the target cell's genome downstream of an endogenous promoter. In contrast, studies on post-translational regulation – e.g. measurements of intracellular protein stability – require a translational fusion between the protein of interest and the reporter luciferase.

Key steps of a reporter experiment

Fig. 1.3 A reporter experiment generally comprises three main steps: (1) cloning, (2) transfection & cultivation of cells, and (3) signal development & detection.

Design of reporter constructs for gene expression studies

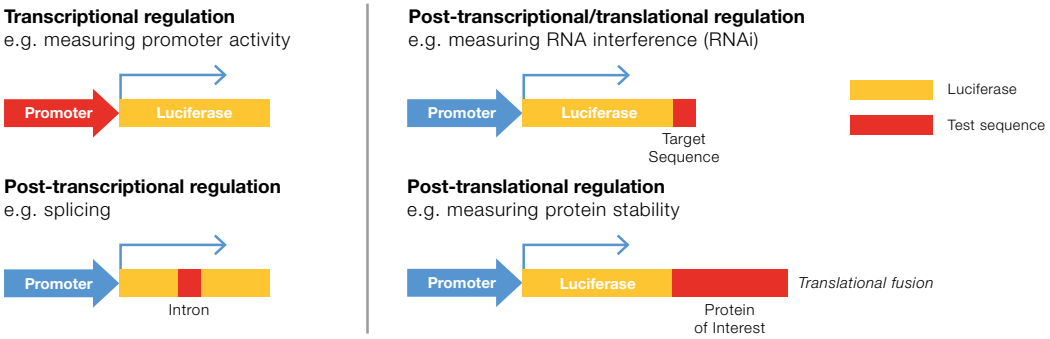


Fig. 1.4 Different reporter construct designs to measure gene expression on various regulatory levels. Sequences to be cloned into respective reporter vectors are highlighted in red.

Promega offers the following types of reporter vectors:

- **Promoterless reporter vectors**
  - – for the analysis at the transcriptional level (e.g. promoter, promoter fragment, and signal transduction studies)
- **Reporter fusion vectors**
  - – for the generation of transcriptional fusions in order to investigate post-transcriptional/translational regulation (e.g. RNAi vectors)
  - – for the generation of tranlational fusions between the protein of interest and the reporter gene
- **Control vectors**

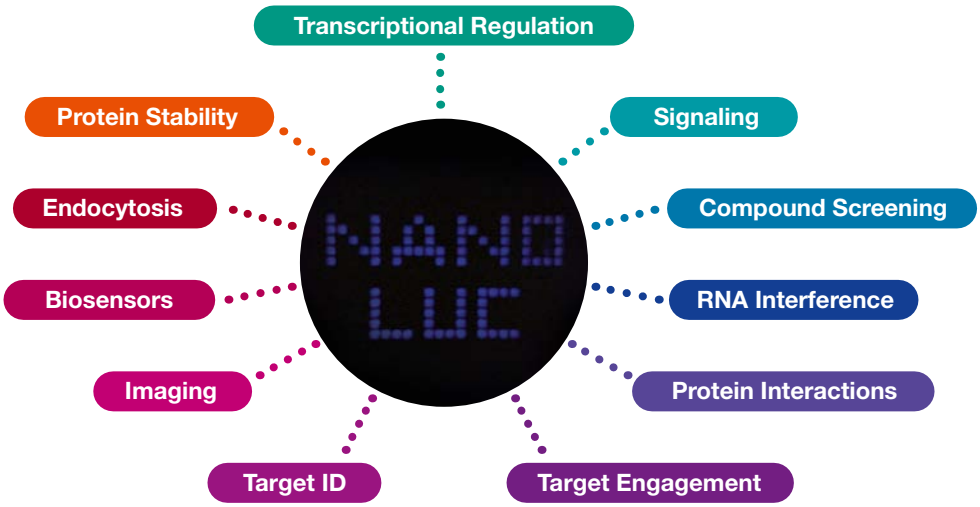
2 Applications of Luciferase Reporters

Introduction – Applications of Luciferase Reporters

Luciferase-based detection systems are broadly applied by scientist from academia as well as pharmaceutical industries due to its unparalleled sensitivity, dynamic range, versatility and ease of use.

Starting from the 1990s, Promega has been continuously developing luciferase-based products and technologies. The development of the highly effective NanoLuc® luciferase (Nluc) opened up novel exciting possibilities and improvements to luminescence applications, including monitoring of protein stability and detection of protein interactions using bioluminescence resonance energy transfer (BRET). The small size and extreme brightness of NanoLuc® luciferase bring exceptional sensitivity to reporter assay applications—allowing detection at low expression levels (e.g., from endogenous promoters or in poorly transfectable primary cells).

Applications of NanoLuc® luciferase



Learn more about the versatility of luciferase reporters in this chapter!

2.1 Promoter Studies with Dual-Luciferase Reporter Assays

Application

Studies on promoter activity, promoter deletion analysis, or analysis of single nucleotide polymorphisms (SNPs) within promoter sequences.

Description

One common application of luciferase reporter genes is the functional analysis of *cis*-acting genetic elements such as promoters ("promoter bashing"). Typically, deletions are made within a promoter region, and their effects on coupled expression of a luciferase reporter gene are determined (Fig. 2.1). Luciferase reporters allow for the functional identification and characterization of promoter and enhancer elements because expression of the reporter protein correlates with transcriptional activity of the reporter gene. In addition to studying the macro level structure of a promoter, investigations on SNPs can also be accomplished by using luciferase reporters.

Principle

For these types of studies, DNA sequences encompassing the full or partial promoter region of interest are cloned into a reporter vector upstream of a luciferase gene (Fig. 2.2). Subsequently, the reporter construct is introduced into cultured cells by standard transfection methods or into a germ cell to produce transgenic organisms. In transient transfection approaches, co-transfection of a second co-reporter that is constitutively expressed from a weak promoter is recommended. This so-called dual-reporter assay is benefical in terms of data reliability and allows for data normalization. In general, dual-reporter assays improve experimental accuracy by reducing data variability caused by differences in transfection efficiency or cell number (see also chapter 6).

Promoter deletion analysis

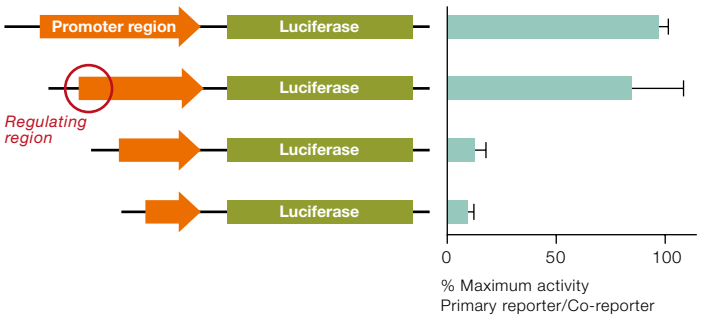


Fig. 2.1 Scheme of a promoter deletion analysis in order to track down regulatory regions within the promoter sequence. Truncated variants of a promoter region of interest are generated and tested with a dual-reporter assay. Normalization with a control reporter is important for the adjustment of experimental variabilities. Altered reporter gene expression upon deletion of a distinct promoter region can be indicative of a regulatory sequence.

Highly optimized reporter vectors

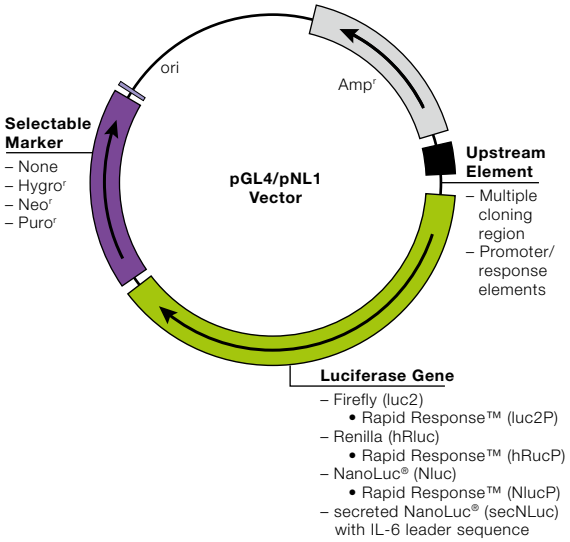


Fig. 2.2 pGL4 and pNL1 plasmids are highly optimized promoterless reporter vectors for regulatory analyses in mammalian cells. Most cryptic regulatory sequences such as consensus transcription-factor binding sites are removed from the vector backbone to avoid co-regulatory effects. The codon usage of luciferase genes is adapted to human ensuring high expression efficiency. Choose *Rapid Response*™ luciferase genes (*luc2P; hRlucP; NlucP*) to measure quick changes in transcriptional activities.

Product Recommendation

Choose dual-reporter assays for transient promoter studies to increase data consistency (see chapter 6). In dual-assays, an experimental reporter (test reporter) and a control reporter (co-reporter) are used. The combinations of Firefly/Renilla, NanoLuc®/Firefly or Firefly/NanoLuc® are possible. As a rule of thumb: NanoLuc®-based dual-reporter assays are considerably more sensitive and surely the right choice when working at physiologically relevant expression levels is an issue.

References

Dieudonné, FX *et al.* (2015) The effect of heterogeneous transcription start sites (TSS) on the translatome: implications for the mammalian cellular phenotype. BMC Genomics 16, 986.

Im, JY *et al.* (2016) DNA damage-induced apoptosis suppressor (DDIAS), a novel target of NFATc1, is associated with cisplatin resistance in lung cancer. Biochim. Biophys. Acta 1863, 40–9.

Shigemoto, T *et al.* (2009) Identification of loss of function mutations in human genes encoding RIG-I and MDA5: implications for resistance to type I diabetes. J. Biol. Chem. 284, 13348–13354.

Yang, HC *et al.* (2009) Isolation of a cellular factor that can reactivate latent HIV-1 without T cell activation. PNAS 106, 6321–6.

Talmud, PJ *et al.* (2005) Determination of the functionality of common APOA5 polymorphisms. J. Biol. Chem. 280, 28215-28220.

Example of a control vector used in dual-assays

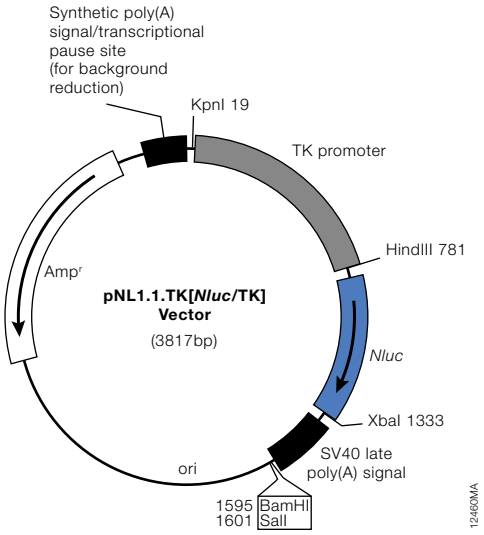


Fig. 2.3 Vector map of a co-reporter plasmid for dual-reporter assays. In this vector, the expression of the reporter gene is regulated by the thymidine kinase (TK) promoter. The TK promoter enables constitutive and moderate expression levels of the control luciferase reporter in many mammalian cells.

Product Box

Possible combinations of luciferases in dual-reporter assays including vector and substrate recommendation

Combination	Experimental reporter vector	Control reporter vector	Substrate
Firefly/Renilla	**Firefly** pGL4.10 [luc2] Vector (Cat.# E6651)	**Renilla** pGL4.74 [hRluc/TK] Vector (Cat.# E6921) pGL4.73 [hRluc/SV40] Vector (Cat.# E6911)	DLR™ (Cat.# E1910) Dual-Glo® (Cat.# E2920)
NanoLuc®/ Firefly	**NanoLuc®** pNL1.1 [Nluc] Vector (Cat.# N1001)	**Firefly** pGL4.53 [luc2/PGK] Vector (Cat.# E5011) pGL4.54 [luc2/TK] Vector (Cat.# E5061)	NanoDLR™ (Cat.# N1610)
Firefly/ NanoLuc®	**Firefly** pGL4.10 [luc2] Vector (Cat.# E6651)	**NanoLuc®** pNL1.1.PGK [Nluc/PGK] Vector (Cat.# N1441) pNL1.1.TK [Nluc/TK] Vector (Cat.# N1501)	NanoDLR™ (Cat.# N1610)

2.2 Signal Transduction – Analysis of Signaling Pathways using Luciferase Reporters

The real power of **reporter gene assays** comes from their ability to be used as cellular readouts for virtually any signaling event. They offer an easy way to quantify signaling pathway activities under various experimental conditions. The activity of many transcription factors has been linked to the induction of distinct signaling pathways or cellular phenotypes (Tab. 2.1). Reporter gene assays measuring signaling events usually involve the cloning of **response element (RE)** repeats into reporter vectors upstream of a minimal promoter. Response elements are short DNA sequences within a promoter region that regulate gene transcription via binding of specific transcription factors. In case of RE reporter vectors, transcription factor binding activates the expression of the reporter gene (Fig. 2.4).

Reporter gene assays for the measurement of signaling pathway activities

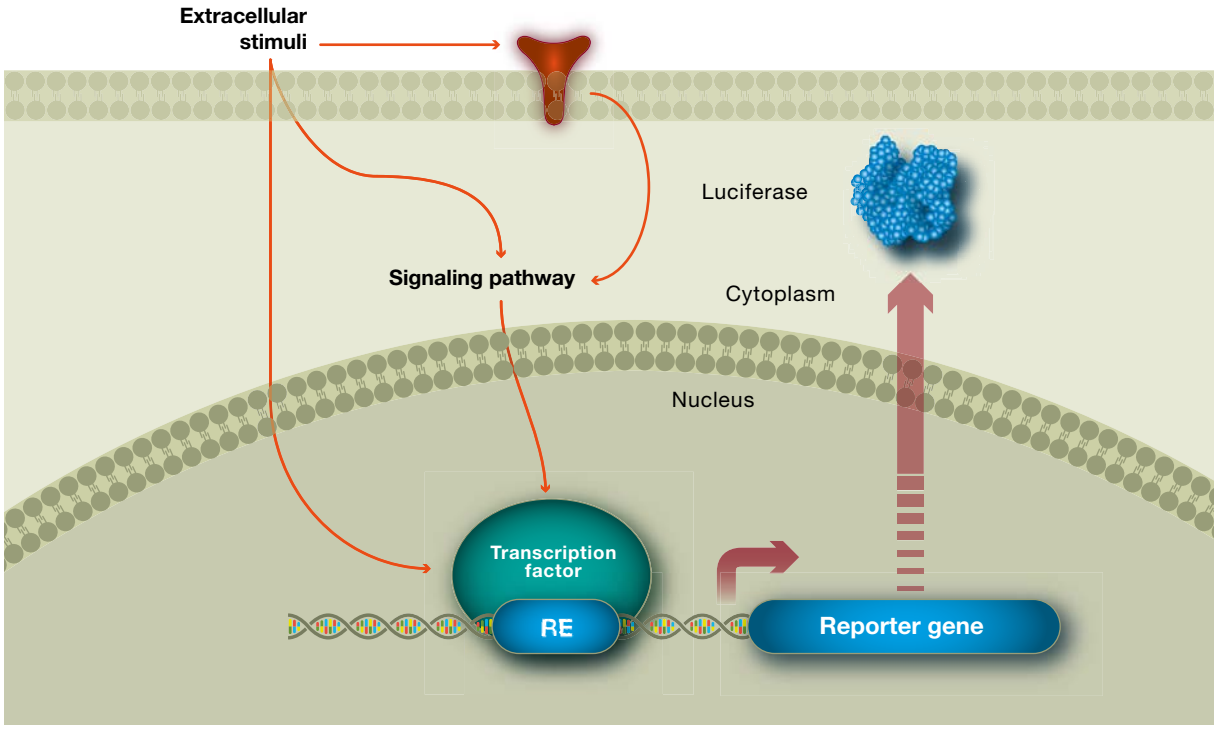


Fig. 2.4 Activation of cellular signaling cascades converge at specific transcription factors (TF). When activated, these TF specifically bind to DNA elements – the so-called response elements (RE) – and initiate transcription of downstream genes and/or the reporter gene.

Promega's reporter vectors are ideally suited for the investigation of signaling pathways. The vector backbone as well as the luciferase genes have been optimized for increased signaling specificity and expression efficiency in mammalian cells (Fig. 2.5). Moreover, the introduction of the so-called **Rapid Response™ Reporters** greatly improved the obtainable signal-to-background ratio by preventing intracellular reporter protein accumulation prior to pathway stimulation. This is achieved by fusing the PEST degradation signal to the C-terminus of the luciferase reporter (Fig. 2.5 and Fig. 2.6). The PEST sequence (40 aa) originates from the murine enzyme ornithine decarboxylase and results in a higher intracellular turn-over of the luciferase protein.

Optimized reporter vectors for the analysis of signaling pathways

(A) Increased signaling specificity and expression efficiency of optimized reporter vectors

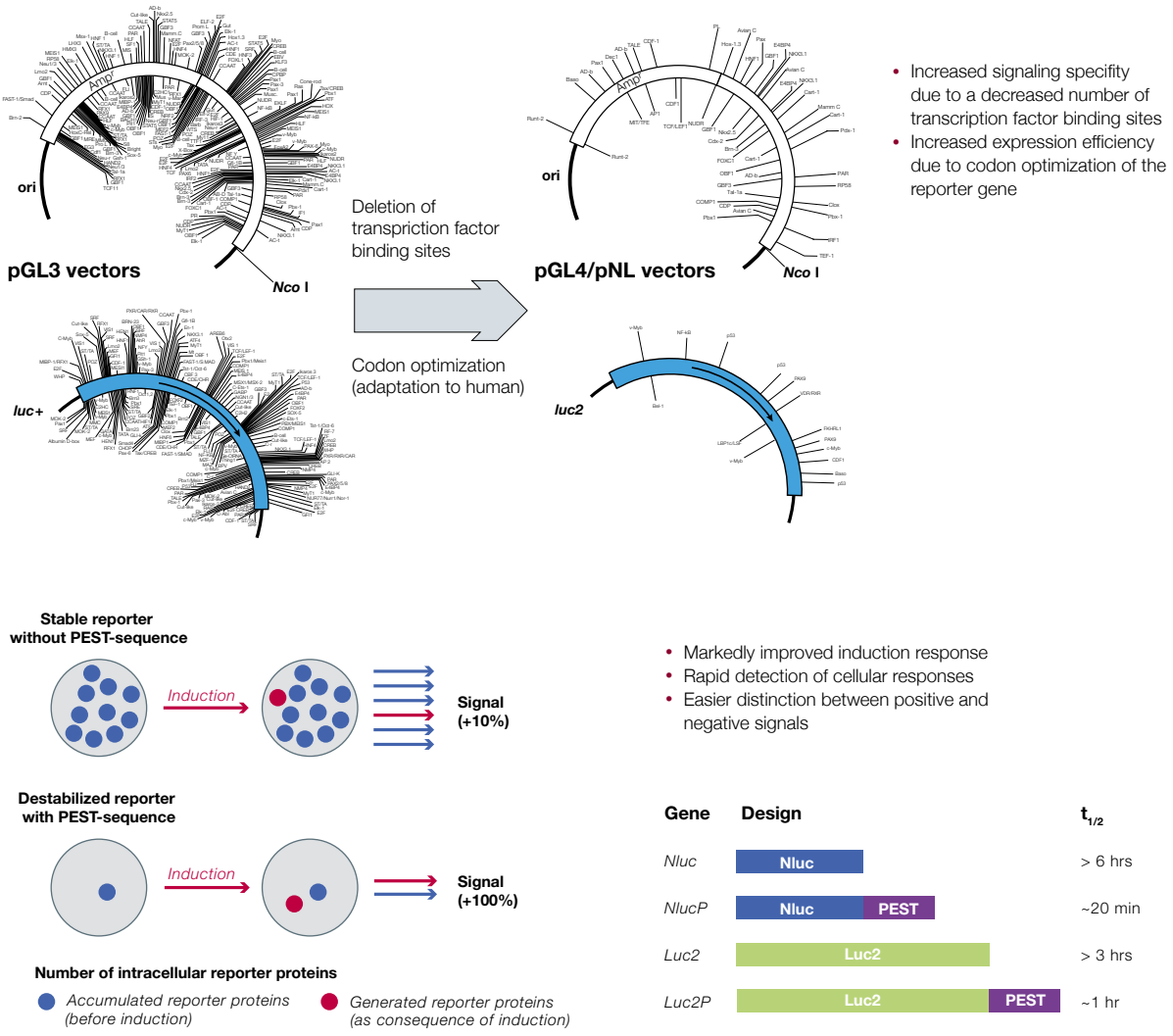


Fig. 2.5 (A) The number of potential transcription factor binding sites within the pGL4 and pNL1 vector backbones and the luciferase gene were considerably reduced in order to increase signaling specificity. Adaptation to human codon usage enhances expression efficiency in mammalian cells. **(B)** Fusion of the luciferase gene to the PEST destabilization sequence shortens the luciferase's cellular half-life ($t_{1/2}$). This prevents accumulation of reporter proteins and thus allows for a higher and faster induction response. The PEST sequence (40 aa) originates from the murine ornithine decarboxylase and was also adapted to human codon usage.

Tab. 2.1: "Ready-to-use" Rapid Response™ signaling vectors and corresponding stable cell lines

Activator/Pathway	Transcription Factor/Response Element (RE)	Nluc	Firefly	Renilla	Stable cell lines
Vectors for GPCR signaling and cellular stress response					
Oxidative Stress	**Nrf2**/Antioxidant response element **(ARE)**	x	x	-	x
cAMP/PKA	**CREB**/Cyclic AMP response element **(CRE)**	x	x	-	x
Calcium/Calcineurin	**NFAT**/Nuclear factor of activated T-cells response element **(NFAT-RE)**	x	x	-	x
NF-κB	**NF-κB**/Nuclear factor κB response element **(NF-κB-RE)**	x	x	-	x
MAP/ERK	**ELK1/SRF**/Serum response element **(SRE-RE)**	-	x	-	x
RhoA (Gα12/13)	**SRF**/Serum response factor response element **(SRF-RE)**	x	x	-	x
DNA Damage/p53	**p53**/p53 response element **(p53 RE)**	x	x	x	-
Endoplasmic Reticulum Stress	**ATF4**/Activating transcription factor 4 response element **(ATF4-RE)**	x	x	-	-
Endoplasmic Reticulum Stress	**ATF6**/Activating transcription factor 6 response element **(ATF6-RE)**	x	x	-	-
Heavy Metal Stress	**MTF1**/Metal regulatory element **(MRE)**	x	x	-	-
Heat shock	**HSF1**/Heat shock element **(HSE)**	-	x	-	-
Hypoxia	**HiF1α**/Hypoxia response element **(HRE)**	x	x	x	x
Xenobiotic Stress	**AhR**/Xenobiotic responsive element **(XRE)**	x	x	-	-
MAPK/JNK	**AP1**/AP1 response element **(AP1 RE)**	x	x	x	x
Myc, PI3K/Akt/MAPK	**Myc/Max**/Myc response element **(Myc)**	x	x	-	-
Androgen Activation in Prostate Cancer	Human PSA promoter **(PSA-long)**	-	x	-	-
Ras/MEK-1	**SRE**/Serum response element (**SRE-RE)**	x	-	-	-
Vectors for cytokine signaling pathways					
JAK/STAT1/2 IFN-α	**STAT1:STAT2**/Interferon stimulated response element **(ISRE)**	x	x	-	-
JAK/STAT3 IL-6	**STAT3:STAT3**/Sis-inducible element **(SIE)**	x	x	-	x
TGF-β	**SMAD3:SMAD4**/SMAD3/SMAD4 binding element **(SBE)**	x	x	-	-
Wnt	**TCF/LEF**/TCF-LEF response element **(TCF-LEF RE)**	x	x	-	-
JAK/STAT5 IL3	**STAT5:STAT5**/STAT5 response element **(STAT5-RE)**	x	x	-	-
Hedgehog	**Gli**/Gli response element **(Gli-RE)**	-	x	-	-
JAK/STAT1 IFN-γ	IFN-γ activation site response element **(GAS-RE)**	x	x	-	x
Multiple	**C/EBP**/C/EBP response element **(C/EBP-RE)**	x	x	-	-
JAK/STAT4 IL12	IRF1 response element **(STAT4-RE)**	-	x	-	-
IL1	Human IL8 promoter **(hIL8)**	-	x	-	-
T cell activation, IL1	Human IL2 promoter **(hIL2)**	-	x	-	-
NGF	Human early growth response factor-1 promoter **(hEGR1)**	-	x	-	-
TGF-β/BMP	Human DNA-binding protein inhibitor ID-1 promoter **(hID1)**	-	x	-	-
STAT3	Human G-CSF **(GCSF)**	-	x	-	-
Notch	**RBP-Jk**/**CBF-1/RBP-$J_κ$** response element **(RBP-Jk-RE)**	-	x	-	-
IL17	Human LCN2 (lipocalin) promoter	-	x	-	-
VEGF2	hVEGFR2 promoter	-	x	-	-
Control Cell Lines					
Control cell line firefly					x
Control cell line NanoLuc®					x

The following Rapid Response™ cloning vectors have been used for the generation of response element (RE) reporter vectors: pNL[NLucP/minP/Hygro] vector (# CS188006), pGL4.27[luc2P/minP/Hygro] (# E8451) and pGL4[hRlucP/minP/Hygro] vector (# CS182201).

Comparison of responses with or without Rapid Response™ reporter

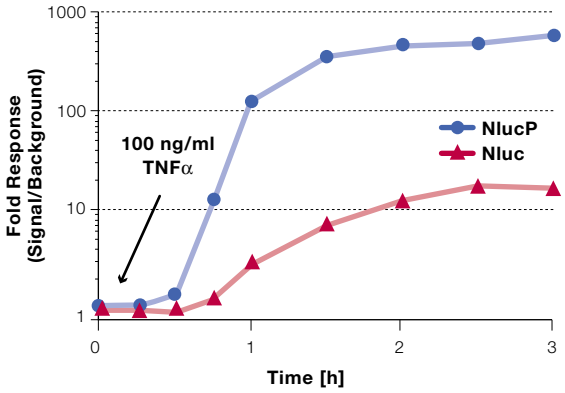


Fig. 2.6 Rapid Response™ reporters increase signal-to-background ratios. The use of destabilized NanoLuc® luciferase (NlucP) as reporter in the NFκB-RE Rapid Response™ reporter plasmid yields a faster and more pronounced response to stimulation with TNFα compared to unmodified NanoLuc® luciferase (Nluc). This experiment was performed with transiently transfected HEK293 cells.

General design of Rapid Response™ vectors for the analysis of signaling pathways

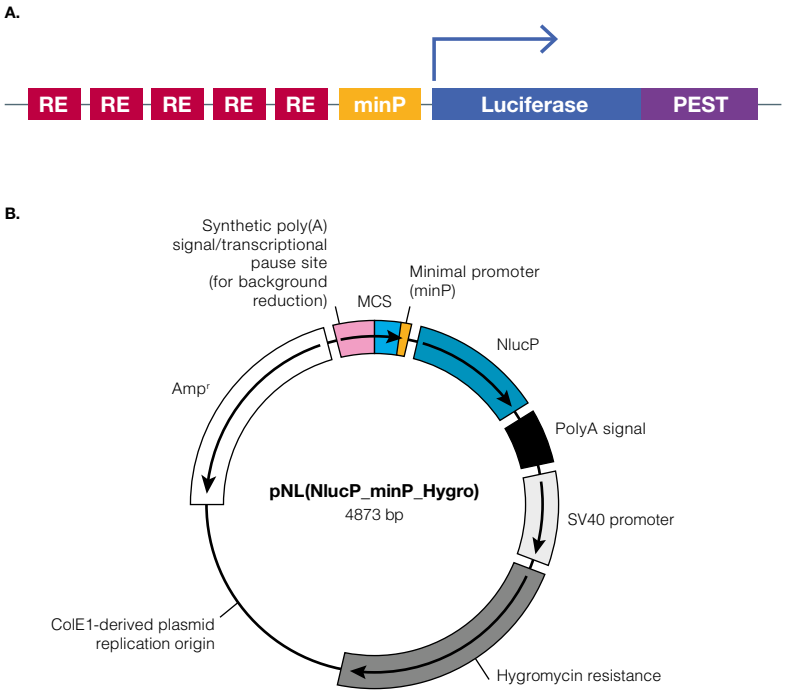


Fig. 2.7 General design of Rapid Response™ vectors. **(A)** Rapid Response™ constructs contain several copies of a response element (i.e. consensus sequence of a transcription factor binding site) upstream of a minimal promoter (minP) as well as a PEST-destabilized luciferase gene. The number of response elements required varies between 1 and 8 and needs to be determined experimentally. Usually, 4–5 copies of the respective response element are sufficient to efficiently initiate transcription. **(B)** Vector map of the reporter cloning vector pNL[NlucP/minP/Hygro] (#CS188006) with minimal promoter and the destabilized NanoLuc® luciferase reporter gene.

References

Promega's **Protocols & Application Guide;** Section: *Bioluminescent Reporters*

GPCR Signal Transduction

Application

Investigation of GPCR signal transduction; screening for GPCR modulators; agonist and antagonist ranking.

Description

The analysis of GPCR (G-protein coupled receptor) signal transduction can be achieved using response element (RE) reporter vectors (see product box below). The stimulation of GPCRs is linked to the activation of specific transcription factors (TFs) which in turn initiate gene expression by binding to their corresponding REs (Fig. 2.8). For example, stimulation of $G\alpha_s$-coupled receptors increases cellular cAMP levels and hence activates the transcription factor CREB (cAMP response element binding protein). Four ready-to-use RE reporter vectors are available for studying GPCR signaling as shown in the product box below.

Principle

Cells are transfected with RE reporter vectors (see product box) and if necessary with a vector encoding the GPCR under investigation. Upon GPCR stimulation, its activity can be followed by measuring luciferase activity.

Reference

Cheng, Z *et al.* (2010) Luciferase Reporter Assay System for Deciphering GPCR Pathways. Curr Chem Genomics. 4:84-91.

Main signaling pathways of GPCR receptors

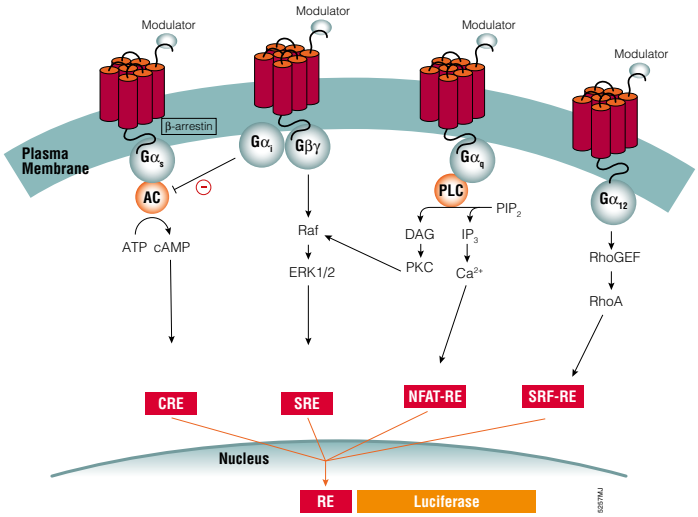


Fig. 2.8 Schematic diagram showing major GPCR signaling pathways. Upon stimulation, **G**α_s coupled receptors activate adenylyl cyclase (AC) resulting in an increase of cAMP; **G**α_i-coupled receptors inhibit AC, and the **G**$\beta\gamma$ subunits activate the ERK pathway; **G**α_q coupled receptors activate phospholipase C (PLC) to produce inositol trisphosphate (IP_3) and diacylglycerol (DAG), which in turn increases intracellular calcium concentration (Ca^{2+}); **G**α_{12}-coupled receptors activate the small G-protein RhoA GTPase. Finally, the activation of G-proteins leads to the activation of transcription factors, e.g. **CREB** (cAMP response element binding protein). Activated transcription factors bind to corresponding response elements (CRE, SRE, NFAT-RE, SRF-RE) and initiate the transcription of downstream genes. **CRE:** **C**yclic-AMP **r**esponse **e**lement; **SRE:** **S**erum **r**esponse **e**lement; **NFAT-RE:** **N**uclear **f**actor of **a**ctivated **T**-cells **r**esponse **e**lement; **SRF-RE:** **S**erum **f**actor **r**esponse **e**lement.

Product Box

Response element (RE) reporter vector for the analysis of GPCR signal transduction

Response-Element	NanoLuc® Vector	Firefly Vector
CRE	pNL[NlucP/CRE/Hygro] (# CS186804)	pGL4.29[luc2P/CRE/Hygro] (# E8471)
SRE	pNL[NlucP/SRE/Hygro] (# CS177601)	pGL4.33[luc2P/SRE/Hygro] (# E1340)
NFAT-RE	pNL[NlucP/NFAT-RE/Hygro] (# CS177602)	pGL4.30[luc2P/NFAT-RE/Hygro] (# E8481)
SRF-RE	pNL[NlucP/SRF/Hygro] (# CS194101)	pGL4.34[luc2P/SRF-RE/Hygro] (# E1350)

The following detection reagents can be used in combination with the reporter vectors for GPCR signaling:
Nano-Glo® Luciferase Assay System (Cat.# N1110); ONE-Glo™ Luciferase Assay System (Cat.# E6110)
Nano-Glo® Dual-Luciferase® Assay (Cat.# N1610) is recommended for dual-assay formats.

Cellular Stress Responses

Application

Detection of cellular stress response via determination of transcription factor activity; toxicologic compound screenings.

Description

Cellular stress response involves a broad range of molecular changes triggered by various stress factors such as extreme temperature, toxic substances, and mechanical damage. By this means cells aim to minimize cell damage and preserve cell integrity. Promega offers a range of pre-designed ready-to-use RE reporter vectors that target pathways key to cellular stress responses (see product box). These constructs can be used individually or in a parallel approach (Fig. 2.9) in order to validate a compound's potency to trigger different cellular stress responses.

Principle

Cells are transfected with RE reporter vectors and subsequently treated with stressor(s) of choice. As a readout for the initiation of distinct cellular stress responses, luciferase activity is derermined.

Identification of the stress response mechanism triggered by tunicamycin treatment using a panel of different RE reporter vectors

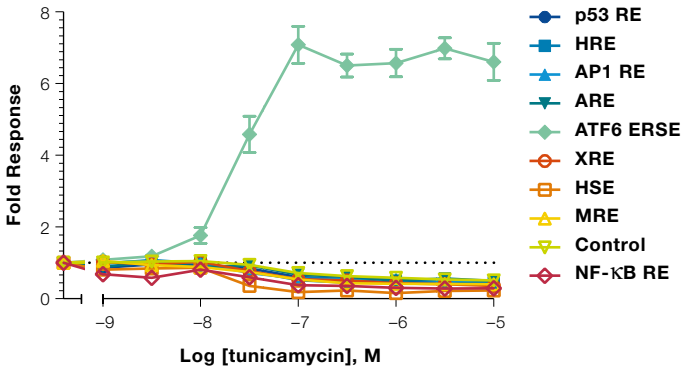


Fig. 2.9 Identification of cellular stress responses initiated upon tunicamycin (a substance that induces ER stress) treatment using a panel of different RE reporter vectors. HEK293 cells were transfected with different RE reporter vectors in a parallel approach. The vector pGL4.13[luc2/SV40] served as negative control.

References

Simmons, SO *et al.* (2009) Cellular stress response pathway system as a sentinel ensemble in toxicological screening.Toxicol. Sci. 111, 202–25.

Landreman, A *et al.* (2013) Novel pGL4 Reporter Vector Panel for Profiling Cellular Stress and Chemical Toxicity. [Promega Website]

Product Box

Response element (RE) reporter vectors to measure cellular stress responses

Stress Pathway	Transcription factor	Response element	Nluc vector	Nluc Cat.#	Firefly vector	Firefly Cat.#
MAPK/JNK	Activation Protein (AP1)	AP1 Response Element (AP1 RE)	pNL[NlucP/AP1-RE/Hygro]	CS177603	pGL4.44[luc2P/AP1 RE/Hygro]	E4111
Inflammation	Nuclear Factor κB (NF-κB)	NF-kB Response Element (NF-κB RE)	pNL3.2.NF-κB-RE [NlucP/NF-κB-RE/Hygro]	N1111	pGL4.32[luc2P/NF-κB-RE/Hygro]	E8491
Oxidative Stress	NF-E2-related factor 2 (Nrf2)	Antioxidant Response Element (ARE)	pNL[NlucP/ARE/Hygro]	CS180902	pGL4.37[luc2P/ARE/Hygro]	E3641
DNA Damage	p53	p53 Response Element (p53 RE)	pNL[NlucP/p53-RE/Hygro]	CS194102	pGL4.38[luc2P/p53 RE/Hygro]	E3651
Endoplasmatic Reticulum Stress	Activating Transcription Factor 6 (ATF6)	ATF6 Response Element (ATF6 RE)	pNL[NlucP/ATF6-RE/Hygro]	CS186805	pGL4.39[luc2P/ATF6 RE/Hygro]	E3661
Hypoxia	Hypoxia-inducible factor 1α (HiF1α)	Hypoxia response element (HRE)	pNL[NlucP/HRE/Hygro]	CS180901	pGL4.42[luc2P/HRE/Hygro]	E4001
Heat Shock	Heat Shock Factor 1 (HSF1)	Heat Shock Element (HSE)	-	-	pGL4.41[luc2P/HSE/Hygro]	E3751

The following detection reagents can be used in combination with RE reporter vectors:
Nano-Glo® Luciferase Assay System (Cat.# N1110); ONE-Glo™ Luciferase Assay System (Cat.# E6110)
Nano-Glo® Dual-Luciferase® Assay (Cat.# N1610) is recommended for dual-assay formats.

Cytokine Signal Transduction

Application

Detection of cytokine signal transduction; compound screenings.

Description

Cytokines are heavily implicated in the regulation of cell proliferation and differentiation. They also serve as mediators of immunomodulating signals. For the analysis of cytokine-mediated signal transduction various reporter vectors are available (see product box). The cytokine-mediated signal transduction triggers the activation of transcription factors, which initiate transcription by binding to their respective response elements (RE). By using appropriate reporter vectors, this activation can be quantified via the detection of luciferase activity.

Principle

The ready-to-use reporter vectors for cytokine-mediated signal transduction (see product box) are transfected into cells and luciferase activity is determined following stimulation with cytokines.

References

Kobayashi, EH *et al.* (2016). Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 7:11624.

Günther, S *et al.* (2014). Molecular determinants of agonist and antagonist signaling through the IL-36 receptor. J Immunol.;193:921-30.

Example for cytokine-mediated signal transduction

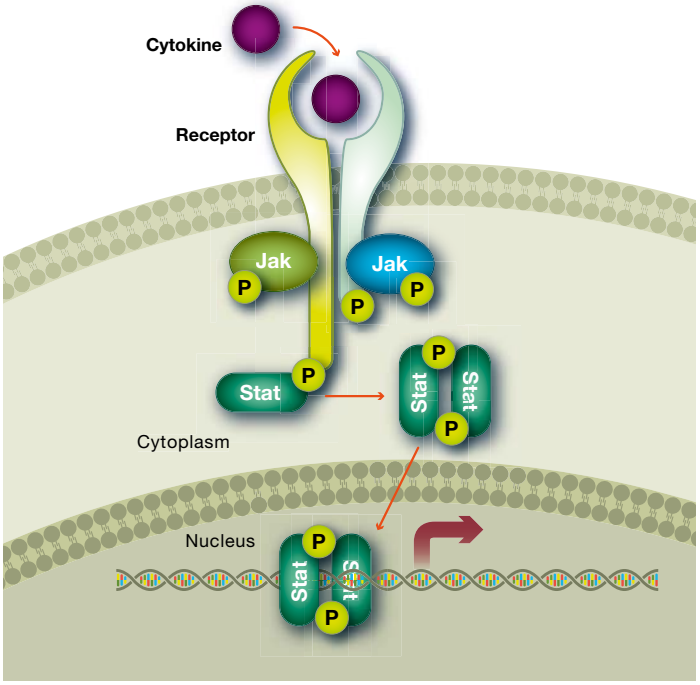


Fig. 2.10 Scheme showing the cytokine-mediated signal transduction via Jak/Stat.

Interleukin-3-mediated activation of STAT5

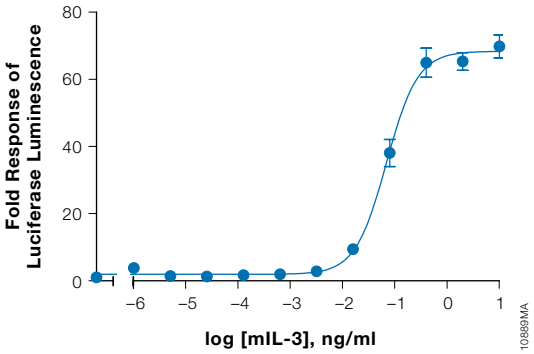


Fig. 2.11 Activation of the transcription factor STAT5 by murine IL-3 (mIL-3). Murine Ba/F3 cells were transfected with reporter plasmid pGL4.52[luc2P/STAT5 RE/Hygro] and stimulated with cytokine mIL-3. Luciferase activity was measured after the addition of the ONE-Glo™ Reagent and recorded with a GloMax® luminometer. The signal was normalized to untreated cells.

Product Box

Ready-to-use reporter vectors for studying cytokine-mediated signal transduction

Cytokine signaling pathway	Transcription factor/ Response Element (RE)	Nluc vector	Nluc Cat.#	Firefly vector	Firefly Cat.#
JAK/STAT1/2 IFN-α	STAT1:STAT2/Interferon-stimulated response element (ISRE)	pNL[NlucP/ISRE/Hygro]	CS190901	pGL4.45[luc2P/ISRE/Hygro]	E4141
JAK/STAT3 IL-6	STAT3:STAT3/Sis-inducible Element (SIE)	pNL[NlucP/SIE/Hygro]	CS189701	pGL4.47[luc2P/SIE/Hygro]	E4041
TGF-β/SMAD3/4	SMAD3:SMAD4/SMAD3/SMAD4 Binding Element (SBE)	pNL[NlucP/SBE/Hygro]	CS177101	pGL4.48[luc2P/SBE/Hygro]	E3671
Wnt	TCF/LEF/TCF-LEF response element (TCF-LEF RE)	pNL[NlucP/TCF/LEF-RE/Hygro]	CS181801	pGL4.49[luc2P/TCF-LEF-RE/Hygro]	E4611
JAK/STAT5 IL3	STAT5:STAT5/STAT5 response element	pNL[NlucP/STAT5-RE/Hygro]	CS180903	pGL4.52[luc2P/STAT5-RE/Hygro]	E4651
Hedgehog	Gli/Gli response element (Gli)		-	pGL4[luc2P/Gli-RE/Hygro]	CS171301
JAK/STAT1 IFN-γ	IFN-γ activation site response element (GAS-RE)	pNL[NlucP/GAS-RE/Hygro]	CS191901	pGL4[luc2P/GAS-RE/Hygro]	CS179301
Multiple	C/EBP/C/EBP response element (C/EBP-RE)	pNL[NlucP/CEBP-RE/Hygro]	CS188003	pGL4[luc2P/C/EBP-RE/Hygro]	CS185201
JAK/STAT4 IL12	IRF1 response element	-	-	pGL4[luc2P/STAT4-RE/Hygro]	CS181501
Interleukin-1	Human IL8 promoter	-	-	pGL4[luc2P/hIL8/Hygro]	CS179401
Interleukin-1	Human IL2 promoter	-	-	pGL4[luc2P/IL-2/Hygro]	CS177201
NGF	Human early growth response factor-1(EGR 1) promoter	-	-	pGL4[luc2P/hEGR1/Hygro]	CS181503
TGF-β/BMP	Human DNA-binding protein inhibitor ID-1 promoter	-	-	pGL4[luc2P/hID1/Hygro]	CS177202
GCS-F/STAT3	Human G-CSF	-	-	pGL4[luc2P/GCSF/Hygro]	CS181505
Notch/CBF-1	RBP-Jk/ CBF-1/RBP-J_k response element	-	-	pGL4[luc2P/RBP-J_k-RE/Hygro]	CS173601

The following detection reagents can be used in combination with above listed reporter vectors:
Nano-Glo® Luciferase Assay System (Cat.# N1110); ONE-Glo™ Luciferase Assay System (Cat.# E6110)
Nano-Glo® Dual-Luciferase® Assay (Cat.# N1610) is recommended for dual-assay formats.

2.3 Compound HTS – Coincidence Luciferase Reporters

Reduce the number of false positive hits as well as the costs for follow-up screens

Application

Compound library screening for the identification of modulators of cell signaling pathways.

Description

Reporter gene assays are commonly applied for compound HTS in order to identify modulators of cell signaling pathways. By using coincidence reporters the number of false positive hits can be substantially reduced which in turn also lowers costs for otherwise required revalidation screens. False positive hits in signal induction assays (gain-of-signal assays) are often based on the stabilization of the reporter signal by direct interaction of the test compound with the reporter luciferase (Fig. 2.13). Coincidence reporters allow for an easier interpretation of screening results since aberrant profiles of two simultaneously measured reporter signals will immediately indicate non-specific hits (Hasson *et al.* 2015).

Principle

Coincidence reporter vectors are bicistronic constructs encoding two reporter proteins: firefly (luc2) and NanoLuc® (NlucP). Transcriptional activation leads to the formation of a bicistronic mRNA that is translated into two separated luciferases in stoichiometric amounts. Translation of the single mRNA into two distinct proteins is ensured by the viral sequence P2A that induces *ribosomal skipping* (Fig. 2.14). The coincidence reporter contains the genes for firefly (*luc2*) and PEST-destabilized NanoLuc® (*NlucP*) luciferase. The PEST sequence prevents cellular accumulation of the very bright NanoLuc® luciferase (Fig. 2.12). Determination of both luciferase activities in a single sample is facilitated by using the homogenous NanoDLR™ detection reagent that is also compatible with automation.

Vector map of a promoterless coincidence reporter vector

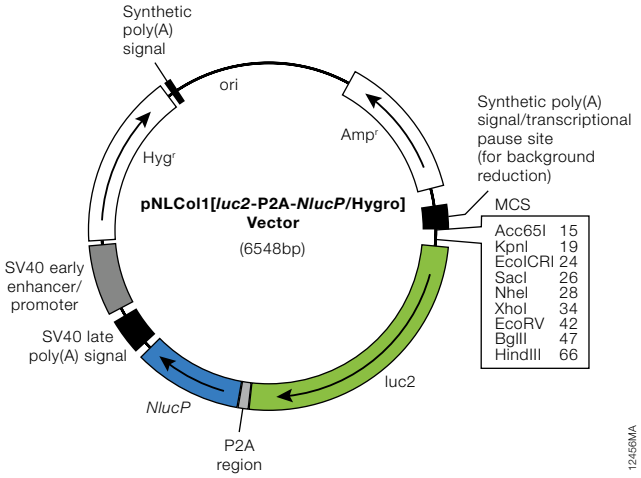


Fig. 2.12 Map of the promoterless coincidence reporter vector pNL-Col1. The *luc2* and *NlucP* genes were codon-optimized for expression in mammalian cells. All pNL vectors contain a minimal number of transcription factor binding sites to reduces unspecific background signals. The promoter sequence or the response element (RE) of interest is cloned into the multiple cloning site (MCS).

Coincidence reporter distinguishes transcriptional activators from compounds stabilizing reporter

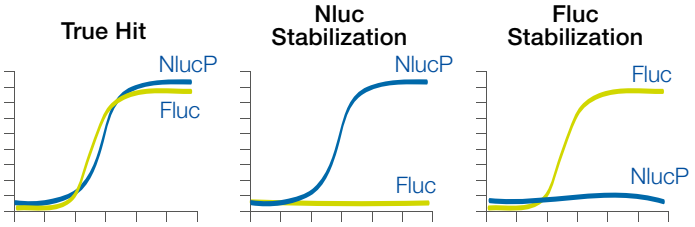


Fig. 2.13 Activity profiles of NlucP and Fluc upon treatment with three putative stimulatory compounds as described in Hasson, SA *et al.* (2015). Congruent activity profiles of both luciferases indicate a true activator. Non-specific hits can be easily identified if the two luciferase reporter signals do not align.

The principle of coincidence luciferase reporters

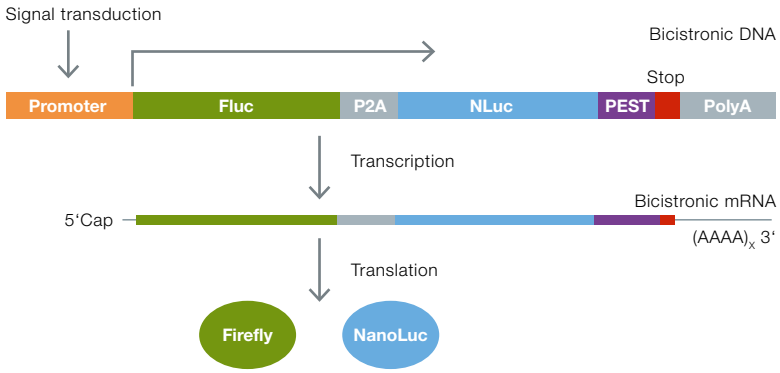


Fig. 2.14 Following compound-induced activation of the promoter of interest that was cloned into a coincidence reporter plasmid, a bicistronic mRNA is transcribed and translated into the firefly and NanoLuc® luciferase. The viral sequence P2A flanked by the two luciferase genes ensures ribosomal skipping, leading to the translation of two independent proteins.

Features

Cost-effective: Reduces the number of false positive hits and otherwise required follow-up screens

Optimized reporter pair: Sensitive, robust and compatible with low copy number/cell

Very good signal/background ratio compared to other reporters

Easy detection due to the homogenous "add-read-add-read" assay format of NanoDLR™ assays; idealy suited for automation

References

Hasson SA *et al.* (2015) Chemogenomic Profiling of Endogenous PARK2 Expression Using a Genome-Edited Coincidence Reporter. ACS Chemical Biology 10, 1188–97.

Product Box

Coincidence reporter vectors

Combination	Description	Coincidence reporter vectors	Substrate (dual)
NanoLuc/Firefly	Promoterless vector	pNLCol1[luc2-P2A-NlucP/Hygro] Vector (Cat.# N1461)	NanoDLR™ Assay Cat.# N1610
NanoLuc/Firefly	Minimal promoter	pNLCol2[luc2-P2A-NlucP/minP/Hygro] Vector (Cat.# N1471)	NanoDLR™ Assay Cat.# N1610
NanoLuc/Firefly	Positive control vector	pNLCol3[luc2-P2A-NlucP/CMV/Hygro] Vector (Cat.# N1481)	NanoDLR™ Assay Cat.# N1610
NanoLuc/Firefly	Positive control vector	pNLCol4[luc2-P2A-NlucP/PGK/Hygro] Vector (Cat.# N1491)	NanoDLR™ Assay Cat.# N1610

2.4 RNA Interference

RNA interference (RNAi) is a naturally occurring mechanism in eukaryotic cells that regulates gene expression at the post-transcriptional level. This regulation is accomplished by short, non-coding RNA molecules which specifically bind to complementary endogenous mRNA sequences. RNAi functions specifically to silence, or deactivate, genes via small interfering RNA (siRNA) and micro RNA (miRNA) that facilitate mRNA degradation by cooperating with the **R**NA-**i**nduced **s**ilencing **c**omplex **(RISC)** and/or by inhibition of mRNA translation (Fig. 2.15).

Micro RNAs are a group of endogenous, non-coding RNA molecules regulating gene expression by binding to the 3'-untranslated region (UTR) of mRNA. **Small interfering RNA** usually refers to synthetically produced RNA that is applied in gene silencing experiments. Nevertheless, siRNA is also formed endogenously by eukaryotic cells. Besides their structure, the main difference between siRNA and miRNA is the degree of complementarity to the respective mRNA target sequence. In general, **siRNA** exhibits 100% complementarity while **miRNA** is only partially complementary and thus can simultaneously be involved in the regulation of mutiple genes.

Mechanism of RNA interference

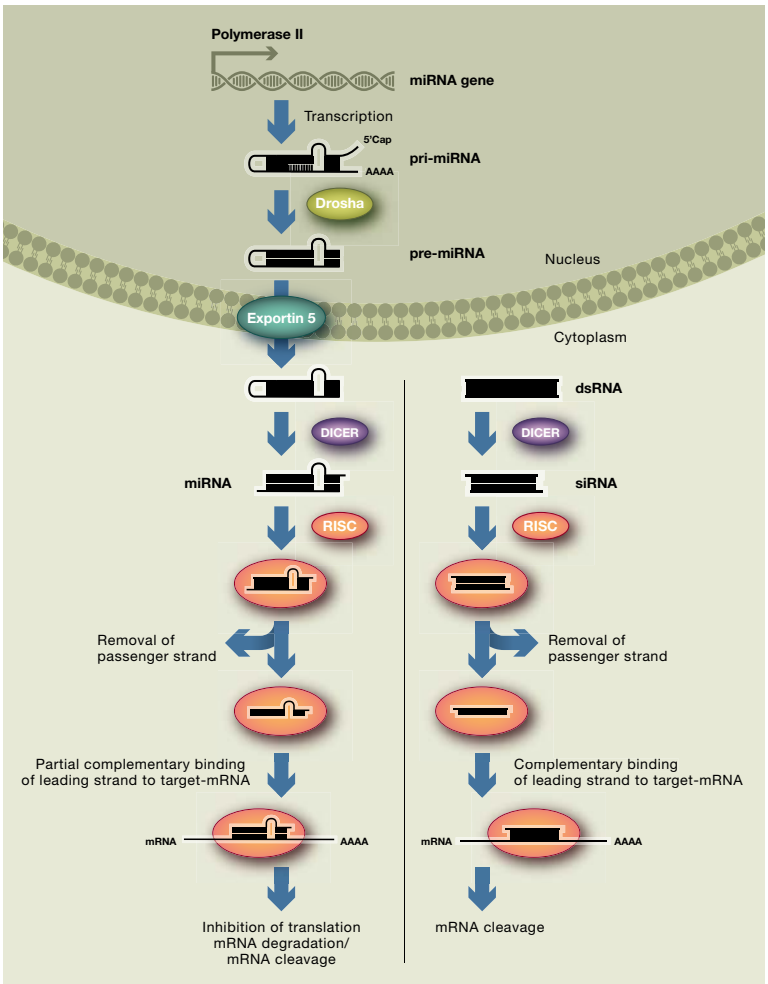


Fig. 2.15 Gene silencing by siRNA and miRNA. In a first maturation process the precursor RNA is cleaved by the enzyme Dicer. In case of siRNA, the precursor is a double-stranded RNA (21-23 base pairs, dsRNA) that is either endogenously transcribed or experimentally introduced into cells. The double-stranded precursor of miRNA – the pre-miRNA (19-25 base pairs) is exported from the nucleus and contains a hairpin loop. The Dicer-processed dsRNA molecules are bound by members of the Argonaut family of proteins to form the RNA-induced silencing complex (RISC) together with other proteins. The guide strand of the dsRNA remains while the passenger strand is degraded. Gene silencing is achieved by blocking the translation of mRNA or by facilitating its cleavage and degradation.

Luciferase reporters for the measurement of RNA interference

Luciferase reporters are used for various purposes in the field of RNA interference (see application box below). For example, in a study by Guo *et al.* (2013) a cellular luciferase reporter assay was established to determine the activity of an endogenous miRNA using the reporter vector pmirGlo (see p. 26). Other studies have used the psiCHECK™-2 reporter vector (see p. 27) to screen for optimal siRNA sequences that facilitate silencing of a certain gene (Takahashi *et al.* 2015).

Application of luciferase reporters for the analysis of RNA interference

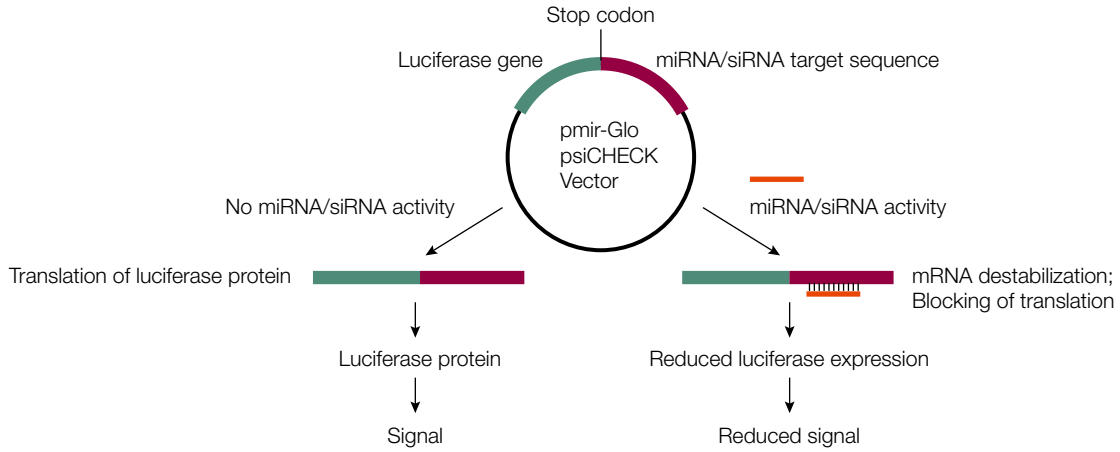


Fig. 2.16 Application of luciferase reporters to analyze RNAi. The miRNA/siRNA target sequence is cloned into a reporter vector 3' of the luciferase gene's translational stop codon. Upon transfection of cells, the luciferase activity is determined. In cells where RNAi is executed a lowered luciferase activity is observed.

Application of luciferase reporters to study RNA interference

- Identification and optimization of siRNA sequences for functional knock-downs
- Detection and quantification of endogenous miRNA/siRNA activity in cells
- Investigation of the transcriptional control regulating endogenous miRNA/siRNA synthesis
- Investigation of miRNA target sequences
- Investigation of transcript stability
- 3'-UTR analysis

Reference

Guo, R *et al.* (2013) Novel microRNA uncovers repression of Let-7 by Gsk-3β. PLoS One.; 8; e66330.

Takahashi, M *et al.* (2015) Normalization of Overexpressed α-Synuclein Causing Parkinson's Disease By a Moderate Gene Silencing With RNA Interference. Mol Ther Nucleic Acids.; 4:e241.

Measuring microRNA Activity with pmir Reporter Vectors

Application

Detection of endogenous miRNA activity; investigation of miRNA binding sites and transcript stability; 3'-UTR analysis.

Description

Luciferase reporters can be broadly applied for the investigation of RNA interference (RNAi). The pmir reporter vectors encode two luciferases and enable quantification of miRNA activity within cells. Two dual vectors can be selected that differ in the type of experimental (NanoLuc® or firefly) and control reporter gene (firefly or Renilla) (see product box below).

Principle

The miRNA target sequence is inserted into the multiple cloning site (MCS), 3' of the experimental luciferase gene. Generally, the 3'-untranslated region (UTR), rarely the 5'-UTR or coding regions of a mRNA, is used as target sequence. The miRNA binds to the target sequence resulting in a reduced translation of the experimental reporter which can be directly monitored. In pmir reporter vectors, the rate of experimental reporter gene transcription is under the control of the human phosphoglycerate kinase promoter (PGK). This weak promoter is beneficial for the quantification of cellular miRNA activity. Furthermore, pmir reporter vectors constitutively express a second luciferase (control reporter). This internal standard allows for the normalization of data in order to account for experimental variabilities.

Reference

Guo, R *et al.* (2013) Novel microRNA uncovers repression of Let-7 by Gsk-3β. PLoS One.; 8; e66330.

Pasqualini, L *et al.* (2015). miR-22 and miR-29a Are Members of the Androgen Receptor Cistrome Modulating LAMC1 and Mcl-1 in Prostate Cancer. Mol Endocrinol.; 29: 1037–1054.

Map of the pmirNanoGlo vector

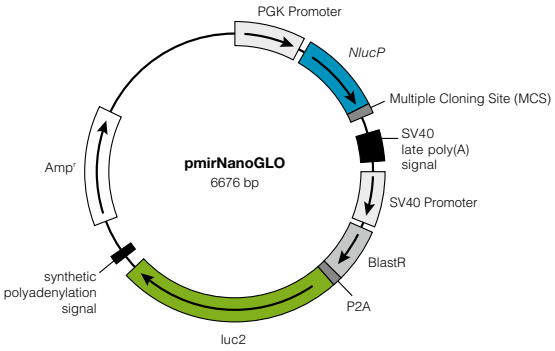


Fig. 2.17 The pmirNanoGlo vector is a dual-reporter vector that can be used to measure miRNA activity. The PKG promoter is applicable to cells from various species, including rat, mouse, human, and yeast. As internal standard, the vector contains the humanized firefly luciferase gene (*luc2*).

Measurement of endogenous miR-21 activity in HeLa cells

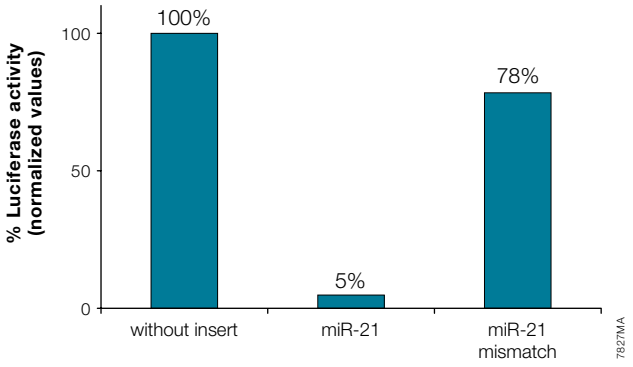


Fig. 2.18 Quantification of miR-21 activity in HeLa cells. Cells were transfected with either one of following three reporter constructs: (1) Insertless control vector, (2) vector with precise complementarity to miR-21, and (3) vector with mismatches to miR-21. Luciferase activity was measured with a dual-reporter assay 24 h post transfection. Data of the experimental luciferase was normalized using the control reporter and expressed relatively to the insertless negative control vector.

Product Box

Combination	Vector for RNA interference	Substrate (Dual)
NanoLuc®/Firefly	pmirNanoGlo (CS194105) (with resistence for stable transfection)	NanoDLR™ Assay Cat.# N1610
Firefly/Renilla	pmir-Glo (Cat.# E1330) (with resistence for stable transfection)	DLR™ Assay Cat.# E1910 Dual-Glo® Assay Cat.# E2920

Measure siRNA Activity with psiCHECK™-2 Reporter Vector

Application

Measurement of RNAi; optimization of siRNA sequences.

Description

The psiCHECK™-2 vector has been developed specifically for siRNA optimization purposes. The efficiency of a siRNA highly depends on its mRNA complementarity – on average, only every fifth siRNA shows an RNAi effect. Beside appropriate predictive algorithms, experimental screenings of numerous siRNAs are key to assure targeting efficiency. The psiCHECK™-2 vector provides a convenient tool for the screening of multiple siRNAs in order to evaluate RNAi efficiency.

Principle

The psiCHECK™-2 vector is a dual-reporter vector encoding two luciferases whereby the huminized Renilla luciferase (hRluc) serves as experimental reporter. The target gene of interest is inserted into the multiple cloning site (MCS), 3' of the translational stop codon of the Renilla luciferase gene. Binding of the siRNA to its target sequence will trigger degradation of hRluc mRNA via RNAi which can be detected by a decline in signal intensity. In case no binding by the siRNA occurs – and thus no RNAi – hRluc is constitutively expressed from the viral SV40 promoter as seen by a constantly increase in luminescence.

References

Takahashi, M *et al.* (2015) Normalization of Overexpressed α-Synuclein Causing Parkinson's Disease By a Moderate Gene Silencing With RNA Interference.Mol. Ther. Nucleic Acids 12, 4:e241.

Yao, T *et al.* (2012) MiR-21 is involved in cervical squamous cell tumorigenesis and regulates CCL20. Biochim. Biophys. Acta. 1822, 248-260.

Product Box

Combination	Vector for RNA interference	Substrate
Renilla/Firefly	psiCHECK™-2 Vector (Cat.# C8021)	DLR™ Assay Cat.# E1910 Dual-Glo® Assay Cat.# E2920 EnduRen™ Live Cell Substrate (Cat.# E6481)*

* EnduRen™ Live Cell Substrate can be used for real-time measurement of RNAi

psiCHECK™-2 vector map

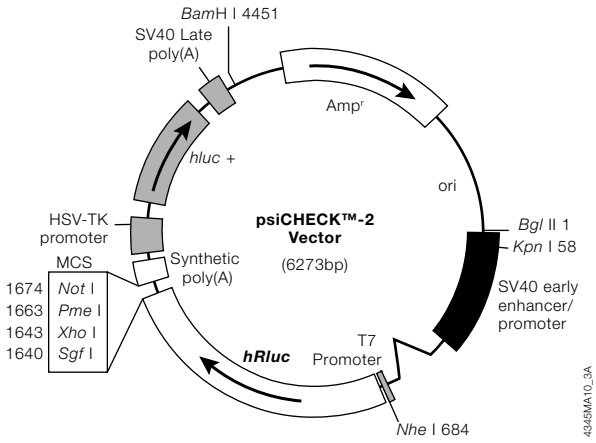


Fig. 2.19 Vector map of the dual-reporter vector psiCHECK™-2. In this vector the humanized Renilla luciferase (hRluc) serves as experimental reporter whose expression is under control of the viral SV40 promoter. The firefly luciferase (hluc +) functions as control reporter enabling data normalization in a lytic endpoint measurement. The Renilla luciferase activity can be determined either in a lytic endpoint measurement with dual-assays or in a kinetic real-time measurement using the EnduRen™ Live Cell Substrate.

Live-cell measurement of RNAi

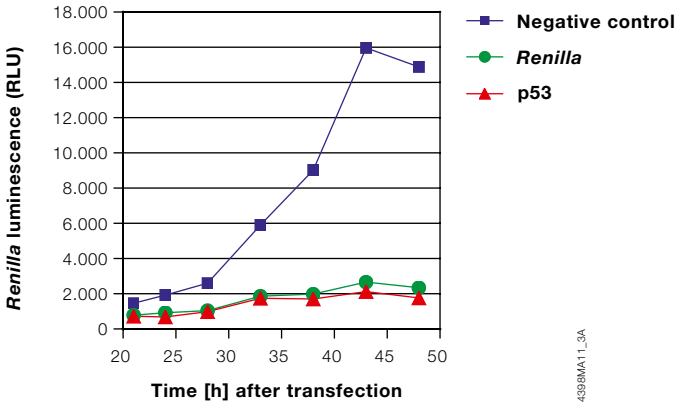


Fig. 2.20 Inhibition of Renilla luciferase expression with RNAi molecules directed against p53 or Renilla luciferase. The human p53 cDNA sequence was cloned into the MCS of the psiCHECK™-2 vector. HEK293 cells were transfected with psiCheck™:p53 and psiLentGene™ vector with integrated small hairpin RNA (shRNA). The shRNA was either complementary to p53 or to the Renilla luciferase mRNA. The psiLentGene™ basic vector – which contains an unspecific 19 bp insert – served as **negative control.** The EnduRen™ Live Cell Substrate (60 µM final concentration) was added 21 hours after transfection and the activity of Renilla luciferase was measured every 3–4 h.

2.5 Protein Stability Sensors Based on NanoLuc® Luciferase

Application

Measuring protein stabilization in response to signaling pathway activation.

Description/Background

The activation of signaling pathways may trigger a cellular response by increasing/decreasing the intracellular half-life ($t_{1/2}$) of coupled regulatory proteins such as transcription factors via their stabilization/destabilzation (Tab.2.2). For example, the transcription factor HIF1α is stabilized during hypoxic conditions prolonging its half-life and subsequent accumulation in the nucleus (Fig. 2.21). This dynamic regulation can be monitored by using a HIF1α-NanoLuc® fusion construct (Fig. 2.22).

Principle

The setup of a protein stability assay requires the generation of a NanoLuc® fusion with the respective regulatory protein of interest (POI). Therefore, plasmids encoding N- and /or C-terminal NanoLuc® fusions are generated by cloning the POI-encoding sequence up- or downstream of the NanoLuc® gene. Subsequently, cells are transfected with the vector(s), the signaling pathway is activated, and NanoLuc® activity is determined using a NanoLuc® Assay Reagent (Fig. 2.22). Ready-to-use NanoLuc® Stability Sensors are available for HIF1α, NRF2 and p53.

References

Robers, M *et al.* Measuring Intracellular Protein Lifetime Dynamics Using NanoLuc® Luciferase. Promega Corporation Web site.

Simmons, SO *et al.* (2009) Cellular stress response pathway system as a sentinel ensemble in toxicological screening. Toxicol. Sci. 111, 202–25.

Tab.2.2 Intracellular half-lifes ($t_{1/2}$) of different transcription factors prior to and following activation of respective signaling pathways.

Target protein	$t_{1/2}$ Normal (minutes)	$t_{1/2}$ Induced (minutes)	Inducer
HIF1α	50	200-250	Hypoxia/mimetics
IκBα	100	5	TNFα/other inflammatory cytokines
p53	20	300-400	Genotoxic stress (e.g., UV/chemical DNA damage)
Nrf2	10-15	30-40	Oxidative stress
β-Catenin	< 60	> 200	Wnt
FOXO	> 300	< 60	Growth factors
PDCD4	300	< 60	Insulin/PI3K
c-Jun	< 60	> 20	Stress
c-Myc	20	300-400	Stress
c/EBP	< 60	> 300	LiCl

Stabilization of HIF 1α in response to hypoxia

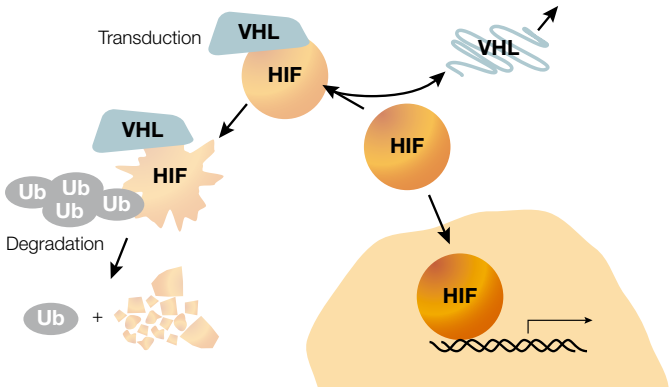


Fig. 2.21 The intracellular stability of HIF1α **(hypoxia-inducible factor 1α)** changes in response to hypoxia. The HIF1α signaling pathway is regulated by the ubiquitin-proteasome system. Under normoxic conditions, the transcription factor HIF1α is repressed by prolyl hydroxylation and von-Hippel-Lindau (VHL)-directed ubiquitination. Ubiquitinated HIF1α becomes destined for proteosomal degradation, resulting in low levels of HIF1α protein and low basal transcription of HIF1α-responsive genes. Upon transition to hypoxic conditions or chemical inhibition of HIF1α prolyl hydroxylation, HIF1α proteins decouple from the ubiquitin/proteasome pathway and accumulate in the nucleus. In turn, transactivation of various HIF1α-responsive genes occurs, resulting in a cellular response to hypoxia.

Measurement of intracellular HIF1α stabilization using HIF1α-NanoLuc® Fusion Proteins

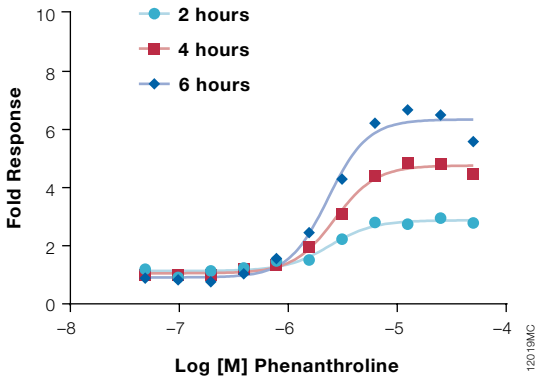


Fig. 2.22 HCT-116 cells were transiently transfected with the NanoLuc® stability sensor plasmid pNLF1-HIF1A [CMV/neo]. The hypoxic conditions were chemically induced by the addition of phenanthroline 20 hours post transfection. NanoLuc® luciferase activity was determined after 2, 4 and 6 hours using the Nano-Glo® Luciferase Assay Reagent and a GloMax® luminometer. Data is given in fold response as ratio of stimulated to non-stimulated sample.

HIF1α-NanoLuc® Stability Sensor

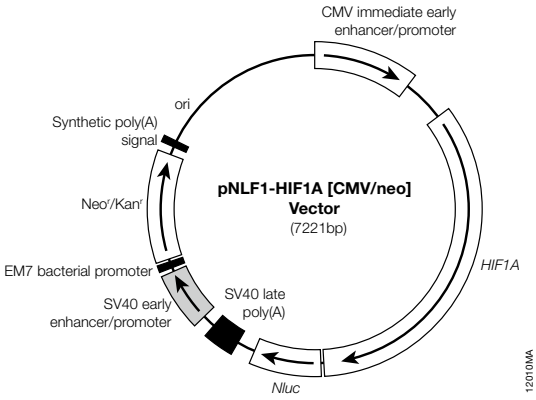


Fig. 2.23 Vector map of the pNLF1-HIF1A [CMV/neo] vector. In this **NanoLuc® Stability Sensor Plasmid**, the HIF1A-encoding sequence is added 5' of the NanoLuc® luciferase gene (*Nluc*).

Product Box

Template vectors for the generation of NanoLuc® stability sensors

Vector description	Cat.#
pNLF1-C[CMV/Hygro]	N1361
pNLF1-N[CMV/Hygro]	N1351

* For additional cloning vectors see vector list in the product overview section.

Ready-to-use NanoLuc® stability sensors for hypoxia, oxidative stress and p53

Stability sensor	Vectors	Cat.#
Hypoxia	pNLF1-HIF1A [CMV/neo]	N1381
Oxidative stress	pNLF1-NRF2 [CMV/neo]/ pKEAP1 [CMV/Hygro]	N1391
p53	p[p53-Nluc/CMV/Neo]	CS179001

Detect the NanoLuc® Luciferase with the Nano-Glo® Luciferase Assay System (Cat.# N1110).

2.6 Protein:Protein Interaction (PPI)

NanoBRET™ PPI Technology

Set up of cell-based PPI assays

Application

Monitoring protein:protein interaction (PPI) in live cells at physiologically relevant expression levels; kinetic measurements of PPI induction or inhibition; small molecule/off-target screenings; peptide library screenings; validation of *in vitro* data; generation of biosensors.

Description

The NanoBRET™ Technology represents a robust, HTS-compatible method for investigating PPIs in living mammalian cells. The method enables dynamic real-time measurements of PPIs at physiologically relevant expression levels and can be applied to monitor both induction and inhibition of a PPI of interest. Assays can be performed in 96- or 384-well formats with low variability and high reproducibility.

Principle

NanoBRET™ Technology is a bioluminescence resonance energy transfer (BRET)-based method using NanoLuc® luciferase as BRET energy donor and HaloTag® protein, labeled with the NanoBRET™ 618 Fluorophore, as energy acceptor to measure the interaction of specific protein pairs. The bright, blue-shifted donor signal and red-shifted acceptor create optimal spectral overlap, increased signal and lower background compared to conventional BRET assays. NanoBRET™ PPI Starter Systems provide the vectors required to generate NanoLuc® luciferase and HaloTag® protein fusions with the target proteins of interest.

References

Machleidt, T *et al.* (2015) NanoBRET- A Novel BRET Platform for the Analysis of Protein:Protein Interactions. ACS Chem Biol. 10:1197-804.

Demont, EH *et al.* (2014) 1,3-Dimethyl Benzimidazolones Are Potent, Selective Inhibitors of the BRPF1 Bromodomain. ACS Med Chem Lett. 5:1190-5.

Wang, J *et al.* (2015) Activation of Rab8 guanine nucleotide exchange factor Rabin8 by ERK1/2 in response to EGF signaling, Proc Natl Acad Sci. 112, 148–53.

Clark, PG *et al.* (2015) LP99: Discovery and Synthesis of the First Selective BRD7/9 Bromodomain Inhibitor. Angew Chem Int Ed Engl. 54:6217-21.

NanoBRET™ Technology for studying PPI dynamics inside living cells

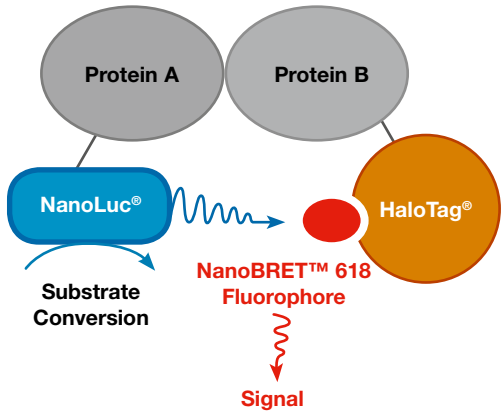


Fig. 2.24 Measuring PPIs in living cells with NanoBRET™ Technology requires the expression of the two interacting proteins of interest (A, B) as fusion proteins with either NanoLuc® luciferase or the HaloTag® protein, respectively. If the interaction partners are in close proximity (< 10 nm), the NanoBRET™ 618 Fluorophore will be excited by the NanoLuc® luciferase via BRET.

Emission spectra: donor/acceptor emission ratio

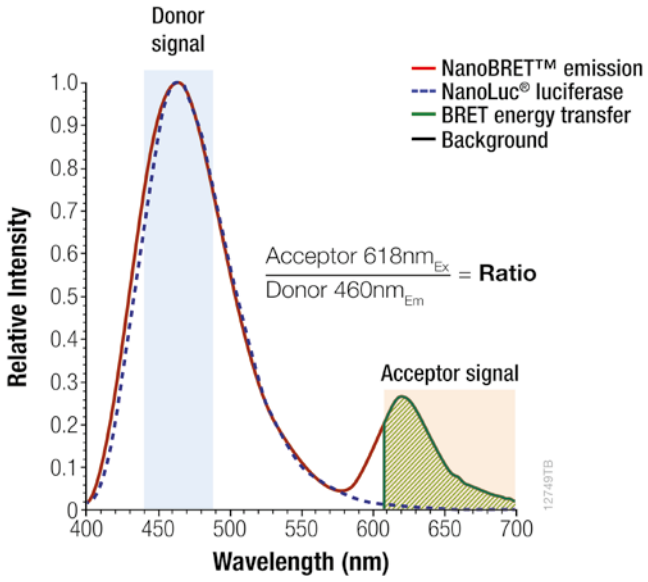


Fig 2.25 Large spectral separation (175 nm) of donor (NanoLuc® Em_{Max} 460 nm) and acceptor signal (HaloTag® NanoBRET™ ligand Em_{Max} 618 nm) assures an optimal signal/background ratio. The signals are recorded in two channels: (1) Donor channel at 460 nm using a band pass filter and (2) acceptor channel at 610 nm using a long pass filter. The BRET ratio is defined as the light signal of the acceptor emission relative to the light signal of the donor emission.

Ligand-induced protein:protein interaction as demonstrated for ß-arrestin 2 and vasopressin receptor 2

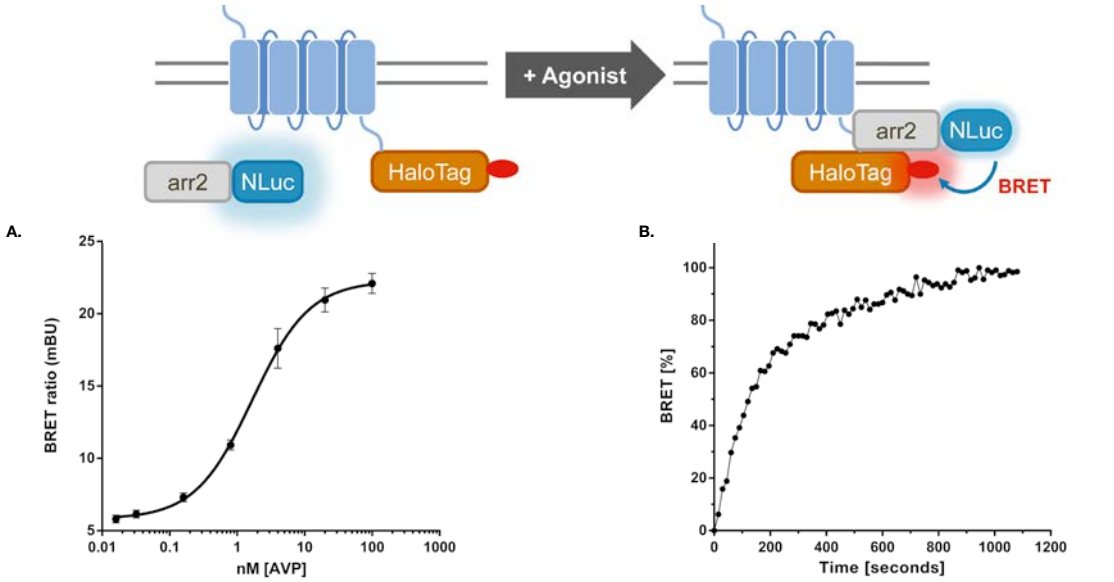


Fig. 2.27 (A) Dose-dependent and **(B)** time-dependent recruitment of ß-arrestin 2 (arr2) to the vasopressin receptor 2 (AVPR2) upon treatment with the peptide hormone arginine vasopressin (AVP).

NanoBRET™ assay workflow

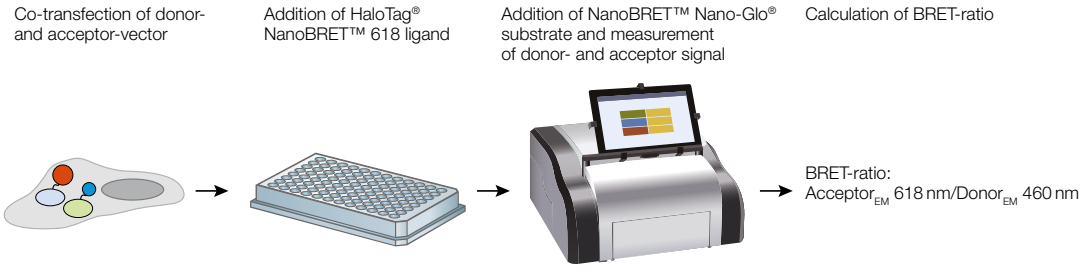


Fig. 2.26 Schematic illustration of the NanoBRET™ PPI assay workflow. Donor and acceptor signals are recorded with a BRET-compatible plate reader such as the GloMax® Discover System using a bandpass filter at 450 nm and a long-pass filter at 600 nm.

Product Box

	Cat.#
NanoBRET™ PPI Starter Kit	N1811, N1821
NanoBRET™ Nano-Glo® Detection System	N1661, N1662, N1663
NanoBRET™ Nano-Glo® Substrate	N1571, N1572, N1573
HaloTag® NanoBRET™ 618 Ligand	G9801
"Ready-to-use" NanoBRET™ assays*	
NanoBRET™ BRD4:Histone H3.3/BRD4:Histone H4 Interaction Assay	N1830/N1890
NanoBRET™ BRD9:Histone H3.3/BRD9:Histone H4 Interaction Assay	N1840/N1900
NanoBRET™ BRPF1:Histone H3.3/BRPF1:Histone H4 Interaction Assay	N1860/N1910
NanoBRET™ cMyc/MAX Interaction Assay	N1870
NanoBRET™ KRas/BRaf Interaction Assay	N1880
NanoBRET™ PPI Control Pair (p53,MDM2)	N1641

* For additional **"ready-to-use" NanoBRET™ assays** refer to www.promega.com/nanobret

NanoBiT® PPI Technology

Set up of cell-based protein:protein interaction assays

Application

Monitoring protein:protein interaction (PPI) in live cells at physiologically relevant expression levels; kinetic measurements of PPI induction or inhibition; small molecule/off-target screenings; peptide library screenings; validation of *in vitro* data; generation of biosensors.

Description

The NanoLuc® Binary Technology (NanoBiT®) is a NanoLuc® complementation assay designed for the sensitive detection of PPIs in living cells. This technology enables setting up real-time or endpoint PPI assays. In contrast to other available complementation assays, NanoBiT® permits the investigation of PPIs at physiological relevant expression levels. Unlike related approaches based on fragmented fluorescent proteins, NanoBiT® complementation is fully reversible and thus allows the measurement of both, protein association and dissociation, over a broad dynamic range.

Principle

The NanoBiT® system is based on two engineered fragments – Large BiT (LgBiT) and Small BiT (SmBiT) – of the intensely bright NanoLuc® luciferase. The BiT fragments have been independently optimized for stability and minimal self-association and are expressed as fusions to target proteins of interest. Interaction of the target proteins facilitates BiT fragment complementation yielding a bright, luminescent enzyme. PPI dynamics can be followed in real-time inside living cells using the Nano-Glo® Live Cell Assay System, a non-lytic detection reagent that contains an optimized cell-permeable substrate.

References

Dixon, A *et al.* (2015) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. ACS Chem Biol. 11:400-8.

Oh-Hashi, K *et al.* (2016) SOD1 dimerization monitoring using a novel split NanoLuc, NanoBit. Cell Biochem Funct. 34:497-504.

Cannaert, A *et al.* (2016) Detection and Activity Profiling of Synthetic Cannabinoids and Their Metabolites with a Newly Developed Bioassay. Anal Chem. 88:11476-11485.

Principle of NanoBiT® for studying PPI dynamics inside living cells

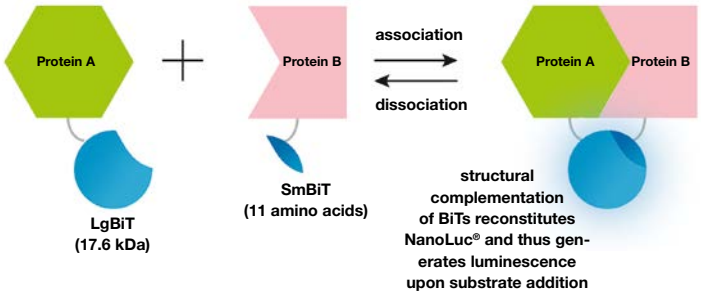


Fig. 2.29 Structural complementation of the two optimized BiT fragments following interaction of protein A and B. For a PPI assay, protein A and protein B are expressed as fusion proteins to LgBiT and SmBiT, respectively. Interaction of fusion partners leads to structural complementation of the BiT fragments, generating a functional enzyme with a bright, luminescent signal.

NanoBiT® assay workflow

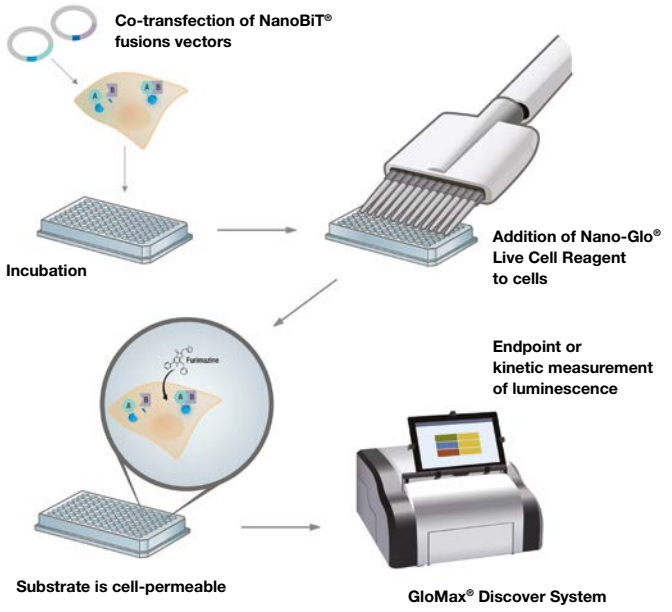


Fig. 2.30 Overview of the NanoBiT® PPI assay workflow.

Inhibition of p53/MDM2 PPI by Nutilin-3

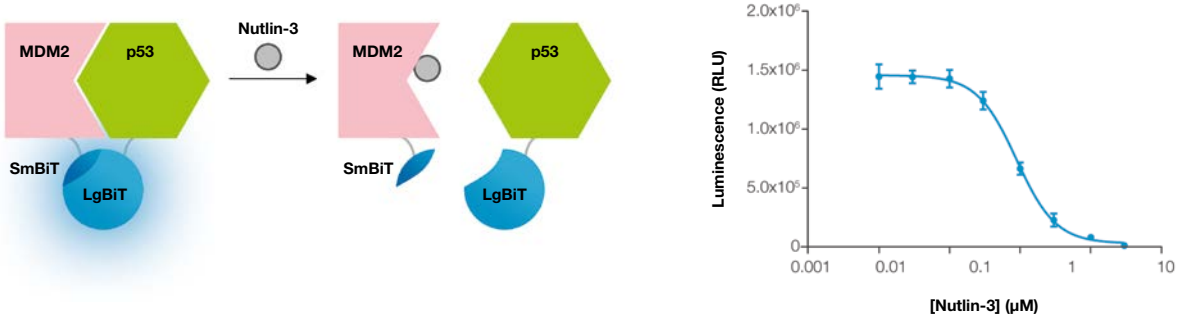


Fig. 2.31 Dose-dependent inhibition of p53:MDM2 interaction by Nutilin-3. Performed in HEK293 cells after transient transfection of LgBiT-p53 and SmBiT-MDM2 fusion constructs.

Detection of androgen receptor (AR) homodimerization using NanoBiT® complementation

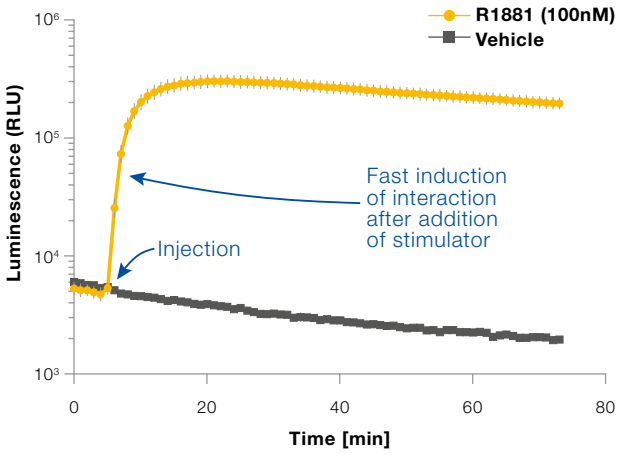


Fig. 2.32 Kinetic measurement of androgen receptor (AR) homodimerization. HEK293 cells were transfected with LgBiT and SmBiT fusions of the AR. Upon addition of the stimulator, R1881, AR homodimerization was followed over time.

High signal intensity of NanoBiT® allows for the measurement of BRAF-CRAF heterodimerization after single copy integration

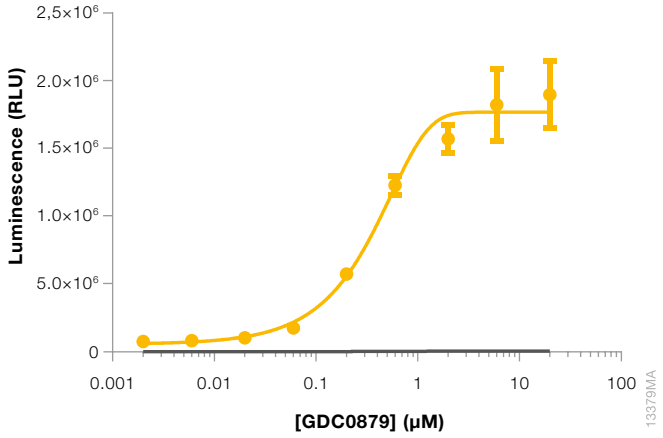


Fig. 2.33 HEK293 cells with single genomic integration of BRAF-LgBiT and CRAF-SmBiT were cultivated under serum deprivation for 2 hours. Dimerization of BRAF:CRAF was induced by the addition of GDC0879. Nano-Glo® Live Cell Assay substrate was added after an incubation time of 4 hours and luminescence was detected. Expression of the integrated split fusions was under the control of a bidirectional CMV promoter.

Product Box

NanoBiT® PPI Starter Kit	Cat.# N2014, N2015
Nano-Glo® Live Cell Assay System	Cat.# N2011, N2012, N2013

2.7 Protein:Ligand Interaction Assays

NanoBRET™ Target Engagement (TE)

Screen-compatible BRET assay for drug development and characterization

Application

Determination of drug candidate binding characteristics to target proteins. Implementation of selectivity screens; target validation, compound optimization. Combinable with phenotypic screens.

Description

NanoBRET™ Target Engagement (TE) is an innovative screen-compatible method to characterize drug candidates. Besides the investigation of drug affinities, the exceptional feature of NanoBRET™ TE is that it also enables determination of a drug's residence time on a given target protein inside living cells. Thus, further important insights into drug dynamics can be gained, which are prerequisite to successful drug optimization.

Readout of NanoBRET™ TE assays

- Drug/target affinity in a cellular context
- Drug cell permeability
- Intracellular drug residence time

Principle

The NanoBRET™ TE Assay is based on the radiation-free bioluminescent resonance energy transfer (BRET) from the intensely bright donor NanoLuc® luciferase onto the spectrally-adjusted acceptor NanoBRET™ 618 fluorophore. This combination allows to set up robust assays with an excellent signal-to-background ratio. The affinity of unknown drugs is determined in NanoBRET™ TE displacement assays (Fig. 2.34) and the duration of the interaction can be monitored in so-called residence time assays (Fig. 2. 35). The acquired data provide a good means to prioritize and validate potential drug candidates.

References

Robers, MB *et al.* (2015) Target engagement and drug residence time can be observed in living cells with BRET. Nat Commun. 6:10091.

Stoddart, LA *et al.* (2015) Application of BRET to monitor ligand binding to GPCRs. Nature Methods. 12(7):661-3.

Arena, S *et al.* (2016) MM-151 overcomes acquired resistance to cetuximab and panitumumab in colorectal cancers harboring EGFR extracellular domain mutations. Sci Transl Med. 8(324):324ra14.

Set up and implementation of NanoBRET™ TE displacement assays for the determination of drug/target affinity

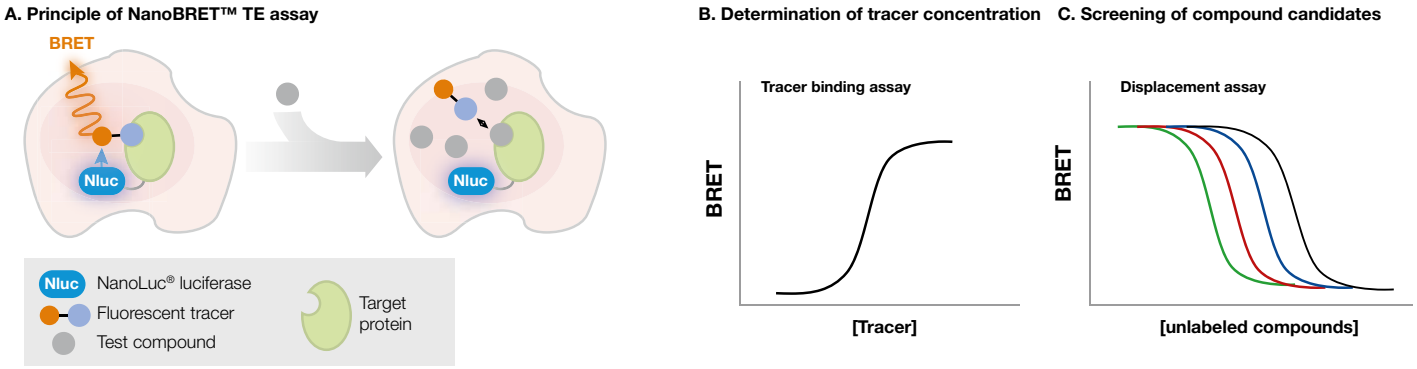


Fig. 2.34 Set up and implementation of NanoBRET™ TE displacement assays. **(A)** Schematic illustration of the NanoBRET™ TE assay principle. **(B)** In a first step, a **binding assay** is performed to determine the optimal tracer concentration. Therefore, the target protein is cellularly expressed as NanoLuc® fusion and the tracer is added at various concentrations. **Tracers** are fluorescently-labeled molecules with known binding affinity to the target protein under investigation. If binding of the tracer to the target protein occurs, a BRET signal will be produced that can be analyzed using a BRET-compatible plate reader equipped with appropriate filters. A tracer concentration in the range of EC_{50}- EC_{80} is usually selected for subsequent displacement assays. **(C)** In a second step, the affinity of non-fluorescent drug candidates is analyzed in a **displacement assay** using the previously determined assay parameters. A constant tracer concentration is applied, while a concentration series of the unlabeled test compounds are added. By this means the IC_{50} value of drug candidates can be easily determined.

Determination of drug residence time

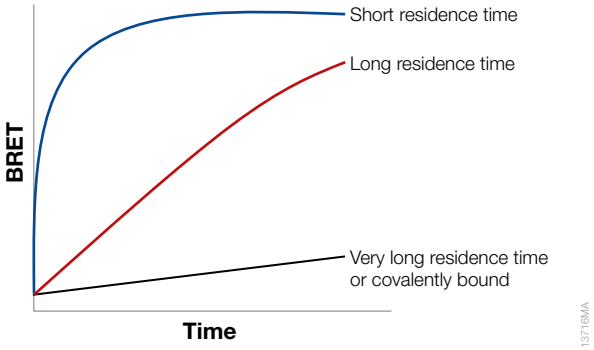
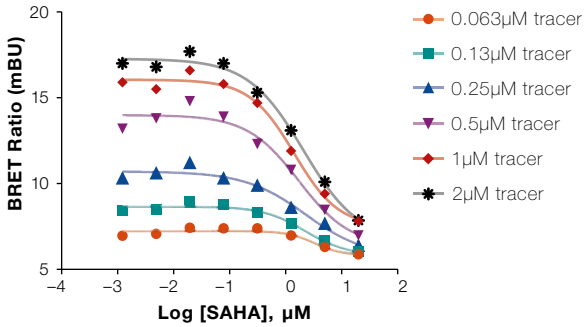


Fig. 2.35 Determination of drug residence time. In a first step, the target protein is loaded with the unlabeled drug candidate followed by the addition of the fluorescent tracer molecule. Displacement of the unlabeled compound by the tracer is monitored by measuring the BRET signal. By this means, the residence time of different drug candidates can be compared.

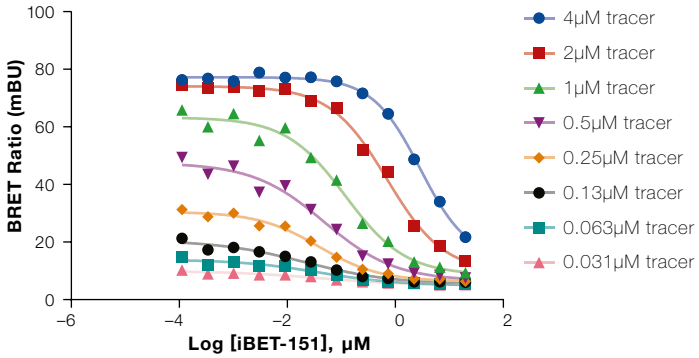
NanoBRET™ TE displacement assays exemplified by HDAC6 and BRD2

A. NanoLuc®-HDAC6 fusion



Tracer Concentration (µM)	2	1	0.5	0.25	0.13	0.063
IC_{50}	2.07	**1.42**	1.71	2.27	2.23	3.32

B. NanoLuc®-BRD2 fusion



Tracer Concentration (µM)	4	2	1	0.5	0.25	0.13	0.063	0.031
IC_{50}	2.9	0.71	0.12	**0.048**	0.034	0.022	0.040	0.043

13695MA

Fig. 2.36 Determination of the intracellular binding affinities of the chemical substances SAHA and iBET-151 using NanoBRET™ TE displacement assays. HEK293 cells, transiently expressing fusion protein **(A)** NanoLuc®-HDAC6 or **(B)** NanoLuc®-BRD2 were mixed with increasing concentrations of the corresponding tracer. Following addition of SAHA or iBET-151 and incubation for 2 hours the BRET signal was determined. At the recommended tracer concentration the following IC_{50} values were obtained: 1.42 µM (SAHA) and 0.048 µM (iBET-151).

Product Box

Ready-to-use NanoBRET™ TE assays for HDACs, BET bromodomains (BRD) family members and kinases:

NanoBRET™ Target Engagement Intracellular HDAC Assay	Cat.# N2080
NanoBRET™ Target Engagement Intracellular BET BRD Assay	Cat.# N2130
NanoBRET™ Target Engagement Intracellular Kinase Assay, K-4	Cat.# N2520
NanoBRET™ Target Engagement Intracellular Kinase Assay, K-5	Cat.# N2500

Products for setting up custom NanoBRET™ TE assays can be found in the product overview.

2.8 HiBiT Protein Tagging System

Monitor translational and post-translational regulation of proteins even at low/endogenous expression levels

Application

Quantification of protein expression, protein stabilization, protein degradation, protein secretion, receptor internalization/recycling and viral infection. Study endogenous biology by CRISPR/Cas9-mediated tagging of endogenous genes with HiBiT.

Description

The HiBiT Protein Tagging System facilitates the set up of easy-to-quantify and highly sensitive protein assays, monitoring regulated changes in cellular protein abundance. HiBiT simplifies protein tagging in live cells, providing a streamlined, antibody-free detection protocol that requires only a luminometer for signal quantification. With a sensitivity down to the endogenous protein expression level and the convenience of a single-reagent-addition step, HiBiT technology opens up a universe of possibilities for researchers studying protein biology.

Principle

HiBiT [HighBiT] is an 11 amino acid peptide tag that can be attached to any protein-of-interest (POI) by either inserting the coding sequence of a POI into a HiBiT fusion vector or by CRISPR/Cas9-mediated gene tagging. The detection of HiBiT-tagged proteins is performed by using reagents that contain the optimized 17.6 kDa LargeBiT (LgBiT)-subunit of the NanoLuc® luciferase and its substrate furimazine. High-affinity binding of HiBiT to the inactive luciferase subunit LgBiT restores the activity of the highly active luciferase enzyme and enables the quantification of HiBiT-tagged proteins at even low expression levels and with an exceptionally broad linear range.

References

Dixon, A *et al.* (2015) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. ACS Chem Biol.11(2):400-8.

Schwinn, MK *et al.* (2017) CRISPR-Mediated Tagging of Endogenous Proteins with a Luminescent Peptide. ACS Chem Biol. *2017 Sep 11.* [Epub ahead of print].

Oh-hashi, K *et al.* (2017) Application of a novel HiBiT peptide tag for monitoring ATF4 protein expression in Neuro2a cells. Biochemistry and Biophysics Reports 12; 40–45.

Principle of the HiBiT Protein Tagging System

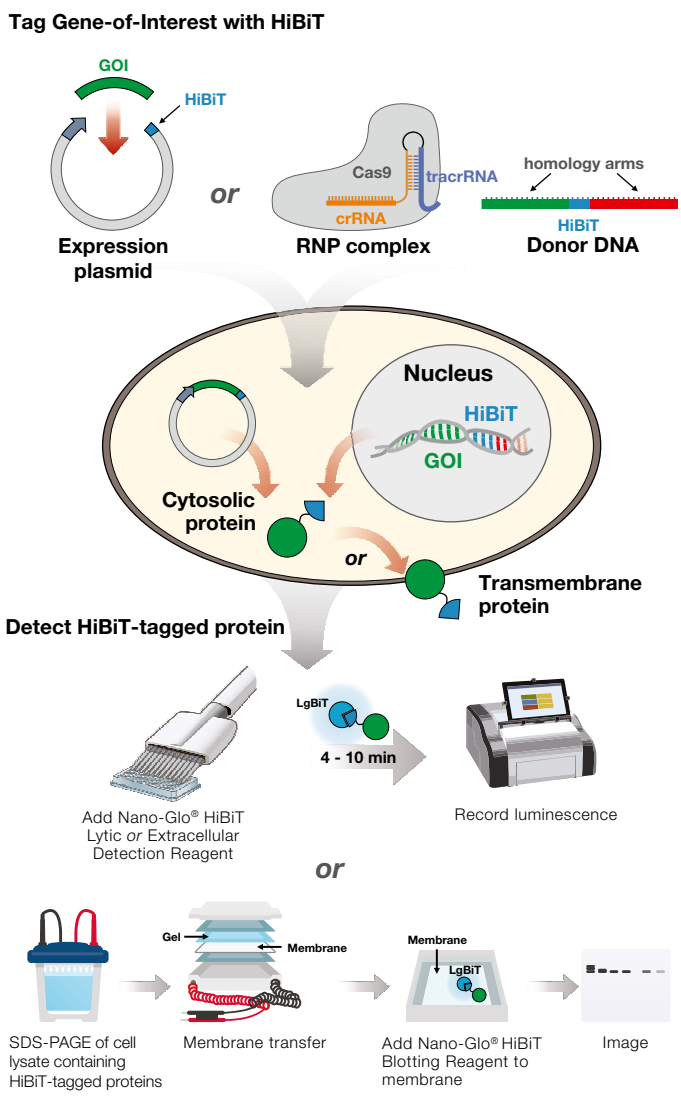


Fig. 2.37 HiBiT tagging can be either achieved by cloning the coding sequence of the protein-of-interest into HiBiT fusion vectors or by genomic insertion of the HiBiT-encoding sequence into the locus of the gene-of-interest (GOI). Expressed HiBiT fusion proteins can be easily quantified in a homogenous add-mix-measure assay using Nano-Glo® HiBiT reagents.

Product Box

HiBiT Fusion Vectors	Cat.# N2361, N2371, N2381, N2391, N2401, N2411
Nano-Glo® HiBiT Lytic Detection System	Cat.# N3030, N3040, N3050
Nano-Glo® HiBiT Excellular Detection System	Cat.# N2420, N2421, N2422
Nano-Glo® HiBiT Blotting System	Cat.# N2410

Precise and sensitive protein quantification over a broad dynamic range

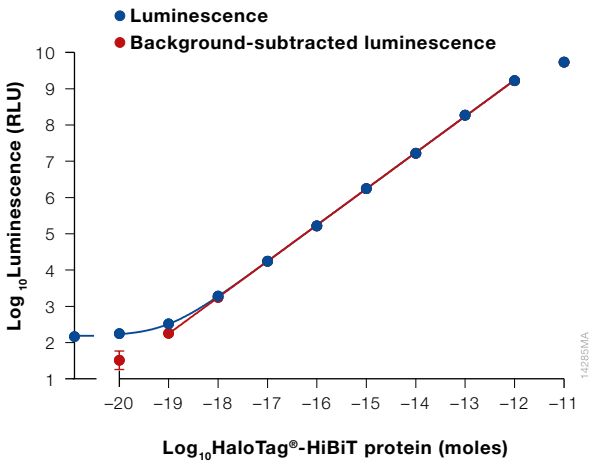


Fig. 2.38 The Nano-Glo® HiBiT Detection System enables accurate quantification of HiBiT-tagged proteins over a broad dynamic range (7 orders of magnitude) and with a detection limit of less than 10^{-19} moles as shown for a 34 kDa fusion protein (HaloTag-HiBiT). The luminescence (blue) and the background-subtracted luminescence (red), are shown.

Measurement of hypoxia-induced stabilization of HIF1α – greatest response at endogenous expression level

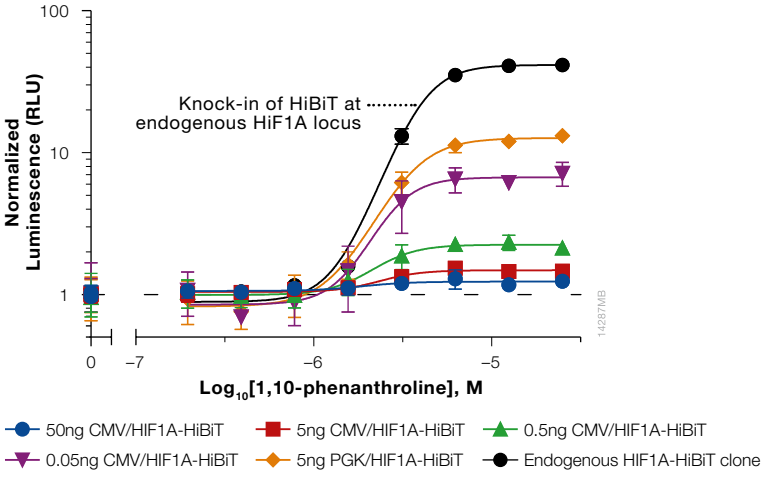
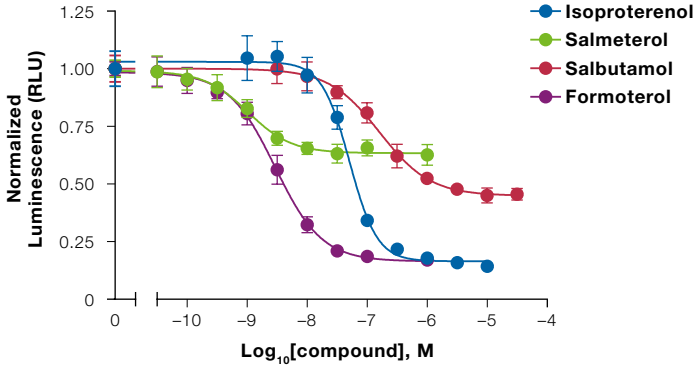


Fig. 2.39 Hypoxia-induced stabilization/accumulation of HiF1α transcription factor. HIF1α-HiBiT was expressed in HeLa cells by either transient transfection of varying amounts of CMV or PGK-promoter-driven expression constructs or by CRISPR/Cas9-mediated HiBiT tagging at the endogenous locus. Upon induction of hypoxia with 1,10-phenanthroline, HIF1α-HiBiT protein expression levels were quantified. Maximal fold-response was detected for HIF1α-HiBiT expressed under the control of the native promoter. Endogenous expression does not only reduces artifacts related to overexpression but also maintains the proper stoichiometry with endogenous binding partners.

Measurement of receptor internalization within minutes



Agonist	EC_{50}	Receptor Remaining on Surface
Isoproterenol	50.9 nM	16%
Salbutamol	161 nM	45%
Salmeterol	1.04 nM	63%
Formoterol	2.92 nM	16%

Fig. 2.40 Internalization of β2-adrenergic receptor (ADRB2) when exposed to different full and partial agonists. The Nano-Glo® HiBiT Extracellular Detection System enables the development of simple, quantitative assays for receptor internalization that save time and eliminate the variability associated with antibody-based methods. The optimized detection reagent results in rapid equilibration with protein receptors, minimizing well-to-well variability and thus can capture rapidly changing biology.

Confirm protein expression after transient transfection or CRISPR /Cas9-mediated integration

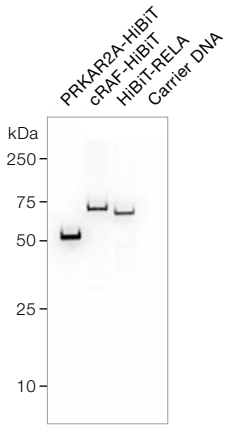


Fig. 2.41 The Nano-Glo® HiBiT Blotting System enables the confirmation of the molecular weight of HiBiT-tagged proteins. When maximal sensitivity is not required, western blots can be imaged within minutes by placing the membrane into the fully reconstituted reagent containing LgBiT protein and substrate.

2.9 Live-Cell Biosensors for cAMP, cGMP and Protease Activity – GloSensor™ Technology

Application

Real-time measurement of changes in cAMP, cGMP levels as well as protease activity in living cells.

Description

The GloSensor™ Technology is based on a genetically altered luciferase that is particularly used for the establishment of intracellular protein sensors, especially in the field of signal transduction. Signal transduction can be measured with high sensitivity, linearity and specificity in living cells allowing for the real-time generation of a kinetic signal transduction profile. The assay is easy scalable to various microplate formats including 96-, 384- and 1536-well plates.

Principle

Protein sensors on the basis of the GloSensor™ Technology use a circularly permutated firefly luciferase in which the wild-type N- and C-termini are tethered by a binding domain or a polypeptide containing a protease cleavage site (Fig. 2.42). The cAMP-GloSensor™ luciferase utilizes the cAMP binding domain of human protein kinase A, the cGMP-GloSensor™ Luciferase contains a cGMP binding domain, and the Caspase 3/7 GloSensor™ luciferase the effector caspase 3/7 recognition peptide DEVD. A conformational change of the GloSensor™ protein is induced either by binding of the second messenger (cAMP or cGMP) to its corresponding binding domain or upon proteolytic cleavage by the caspase 3. Consequently, luciferase activity is restored whereby the resulting bioluminescene is directly proportional to the amount of second messenger or protease activity.

Schematic illustration of the GloSensor™ Technology

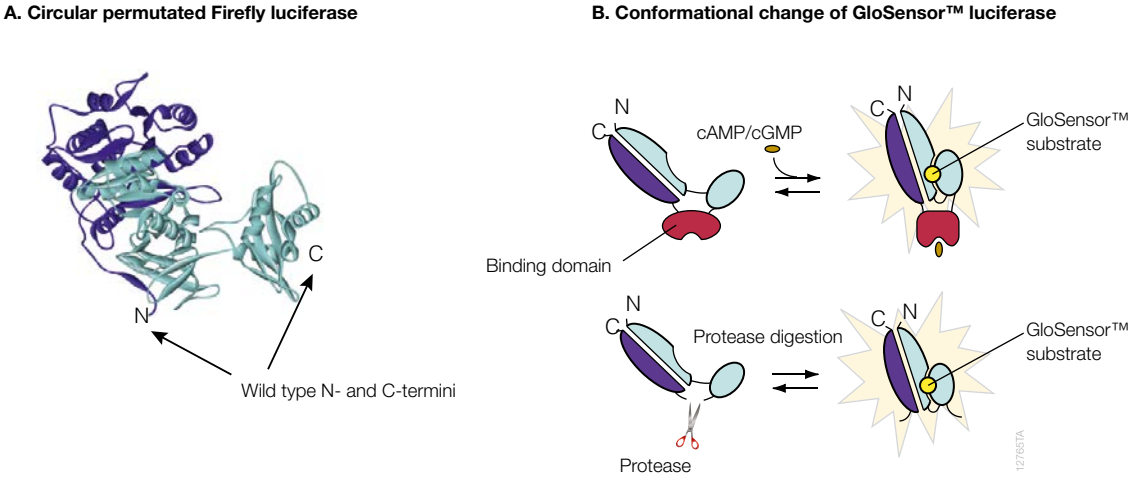


Fig. 2.42 The GloSensor™ Technology is based on a circular permutated firefly luciferase that serves as an intracellular bioluminescent protein sensor. **(A)** The protein sensors are generated by linking the wild-type N- and C-termini of the luciferase with a second messenger binding domain or a polypeptide containing a protease recognition site. **(B)** A conformational change of the GloSensor™ Protein upon second messenger binding or proteolytic cleavage restores the luciferase's catalytic activity.

Real-time measurement of changing cGMP level in cells

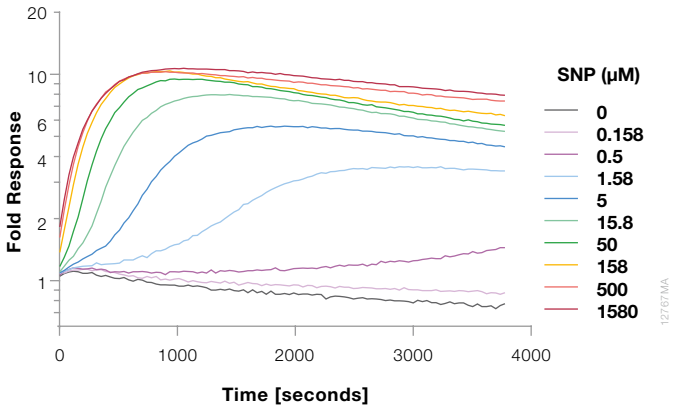


Fig. 2.43 HEK293 cells were transiently transfected with the cGMP sensor (pGloSensor™-42F cGMP Plasmid) and treated with sodium nitroprusside (SNP). Sodium nitroprusside is a nitrogen monoxide donor, which activates the endogenous soluble guanylyl cyclase. The cGMP change was determined in real-time using the non-lytic GloSensor™ Reagent.

The caspase 3/7 GloSensor™ luciferase

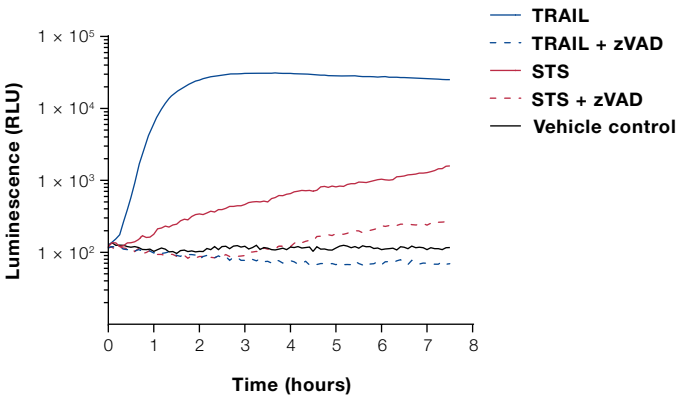


Fig. 2.44 D54-MG glioma cells were stably transfected with the pGloSensor™-30F DEVDG vector and treated with either an extrinsic or an intrinsic inducer of apoptosis. At time point zero, the cells were treated with 200 ng/mL TRAIL or 1 µM staurosporine (STS), respectively. In a parallel approach, cells were treated with a combination of the respective inducer and 20 µM of the pan-caspase inhibitor, zVAD-FMK, in order to verify specificity. By using the non-lytic GloSensor™ Reagent the caspase 3/7 activity was determined by reading the luminescence every 5 minutes.

Areas of Application

- High-throughput analyses of agonists, antagonists, inverse agonists and allosteric modulators
- Pharmacologic characterization of substances/compounds
- Real-time quantification of the intracellular cAMP/cGMP concentration; determination of Gs- and Gi-coupled receptor biology
- Measurement of protease activity

References

Fan, F *et al.* (2008) Novel genetically encoded biosensors using firefly luciferase. ACS Chem. Biol. 3, 346–51.

Binkowski, BF *et al.* (2009) Live-cell luminescent assays for GPCR studies. Gen. Eng. Biotech. 29, 30–1.

Binkowski, BF *et al.* (2009) Engineered luciferases for molecular sensing in living cells. Curr. Opin. Biotech. 20, 14–8.

Wigdal, S *et al.* (2008) A novel bioluminescent protease assay using engineered firefly luciferase. Curr. Chem. Genomics 1, 94–106.

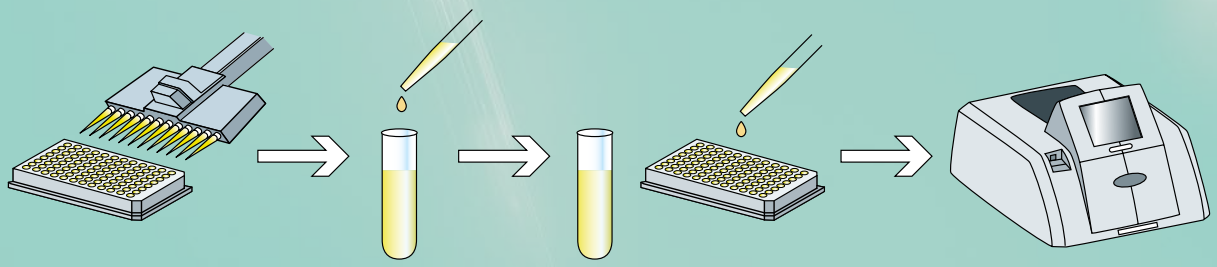
Fan, F *et al.* (2007) Bioluminescent assays for high-throughput screening. Assay Drug. Dev. Technol. 5, 127–136.

Product Box

cAMP detection	Cat.#
GloSensor™ cAMP HEK293 Cell Line	E1261
pGloSensor™-22F cAMP Plasmid	E2301
GloSensor™ Reagent	E1290
cGMP detection	
GloSensor™-40F cGMP HEK293 Cell Line	CS182801
pGloSensor™-40F cGMP Plasmid	CS178901
pGloSensor™-42F cGMP Plasmid	CS177001
GloSensor™ Reagent	E1290
Protease activity detection	
pGloSensor™-30 DEVDG Caspase Plasmid	CS182101
GloSensor™ Reagent	E1290
Template plasmid for the generation of custom protein sensors to detect protease activity	
pGloSensor™-10F Linear Vector	G9461

3 Transfection Reagents

A simple transfection protocol without the necessity to exchange medium



Seed adherent cells one day prior to transfection. Cells should be 50-80 % confluent on the day of transfection. Seed suspension cells on the day of transfection.

Dilute DNA in media; add FuGENE® or ViaFect™ Reagents and incubate.

Add lipid:DNA mixture to cells. Neither medium exchange nor reduction of serum is required.

Implementation and measurement of luciferase activity after 24-48 hours.

Fig. 3.1 Schematic illustration of the transfection procedure using FuGENE® or ViaFect™ Transfection Reagents.

The transfection reagents FuGENE® or ViaFect™ allow for high-efficiency transfection of a wide range of cell types without compromising cell viability. An easy protocol without the need for medium exchange simplifies your experimental workflow and reduces cross-experimental variability.

Transfection of Cells

Transfection is a key step in the course of a reporter experiment. Most critical is the balancing act of maximizing efficient DNA transfer into the cell's nucleus while minimizing impairment of cell viability. There are various methods available for the introduction of foreign DNA into a eukaryotic cell, ranging from non-viral methods, such as physical or chemical transfection, to viral-based transfection methods. The use of cationic lipids in a chemical transfection approach is commonly applied due to its simple handling and high transfection efficiency. Cationic lipids consist of a positively charged head group which is connected to a long lipid tail via a linker (Fig. 3.2). As amphiphilic molecules, they bind to negatively charged DNA. Compared to other techniques, this method has a wide cell compatibility and lower cell toxicity.

Cationic lipids

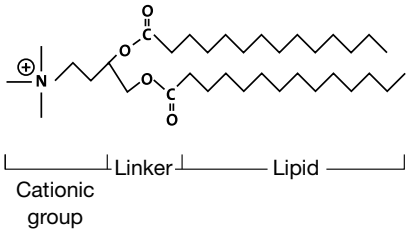


Fig. 3.2 General structure of a synthetic cationic lipid.

Transient or stable transfection?

In case of transient transfection, the transfected extrachromosomal DNA gets lost after a few mitotic cell divisions. In stably transfected cells, the foreign DNA is permanently integrated into the host cell's genome and hence passed on to the next generations. The relatively time- and cost-effective establishment of a stably transfected cell line can pay off when constantly recurring experiments are planned – e.g. large-scale screens that require low variances. For analyses of various DNA constructs – e.g. test deletion variants of a gene – transient transfection is generally applied.

Factors supporting successful transfection

Transfection efficiency depends on a myriad of factors and is cell type-specific. Published transfection protocols can serve as a good starting point for adaptations to a distinct cell line in order to yield high transfection efficiency and statistically relevant data.

There are some fundamental factors that considerably contribute to success:

- Cells should actively divide, they should regularly be passaged and be fed with fresh cell culture medium.
- Cells must be free of contaminations, e.g. mycoplasma or yeast.
- Ideally, cells are about 50-80% confluent on the day of transfection.
- Quality and purity of transfected DNA is of major importance. The A_{260}/A_{280} ratio should lie between 1.7 and 1.9.

3.1 FuGENE® 6/FuGENE® HD Transfection Reagent

Application

Transfection of adherent cells, suspension cells, primary cells, and stem cells. Tested for a variety of mammalian cells and insect cells.

Description

FuGENE® 6 and **FuGENE® HD** are non-liposomal transfection reagents that were especially developed for cell transfection. The transfected plasmid DNA forms a complex with a mixture of lipids enabling uptake by the target cell. The protocol is easy and convenient (Fig. 3.1), an exchange of cell culture medium is not required. This is particularly beneficial when using multi-well plates since the experimental reproducibility is certainly higher without media aspiration. FuGENE® Transfection Reagents are serum-compatible, which means cell culture conditions do not need to be adjusted.

Numerous cell lines have already been successfully transfected with high efficiency using FuGENE® 6 without the need for elaborate optimization procedures. The advanced transfection reagent FuGENE® HD is especially suited for hard-to-transfect cell lines (e.g. primary cells). A comparison of both reagents is shown in Tab. 3.1.

Features

- High transfection efficiency with low toxicity
- Simple implementation without medium exchange
- Serum-compatible
- Quality product: each lot is tested
- Ideally suited for highly sensitive luciferase assays
- Detailed online database with transfection protocols for various cell lines and cell types

References

Mentioned in hundreds of publications!

Product Box

FuGENE® 6 Cat.# E2691, E2692, E2693
FuGENE® HD Cat.# E2311, E2312

Comparison of different transfection reagents

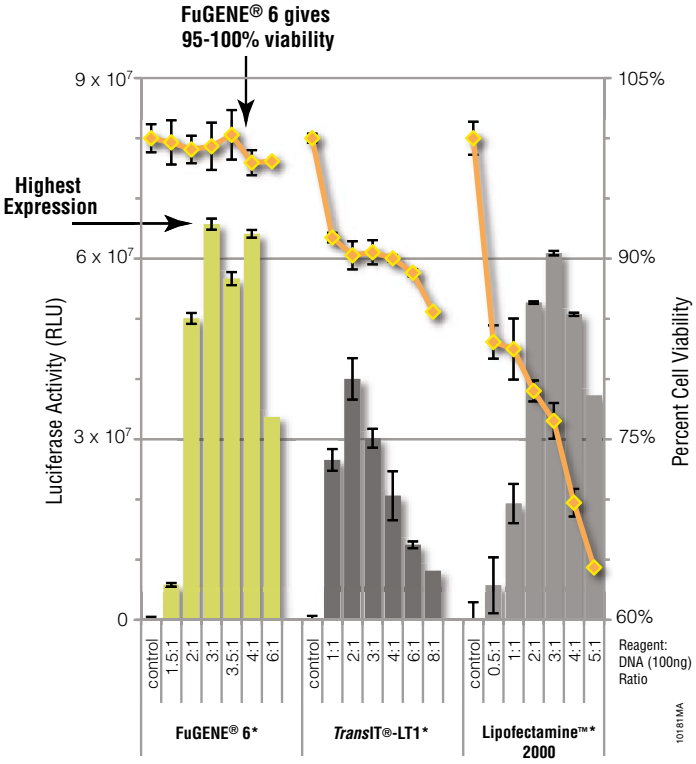


Fig. 3.3 Comparison of FuGENE® 6 with transfection reagents of other suppliers. HEK293 cells were transfected with three lipid-based transfection reagents at different lipid/DNA ratios using a luciferase reporter construct. Luciferase activity and cell viability were determined. FuGENE® shows higher transfection efficiency at considerably lower toxicity.

* FuGENE is a registered trademark of Fugent, LLC. Lipofectamine is a trademark of Life Technologies, Inc. and TransIT is a registered trademark of Mirus Bio, LLC

Tab. 3.1 Comparision of FuGENE® HD and FuGENE® 6 applicability

Requirement	FuGENE® HD	FuGENE® 6
Transfection of model cell lines	****	****
Difficult to transfect cell lines	****	**
Transfection of primary cells	***	*
Non-toxic reagents	***	****
Easy of use	***	****
Protein production	****	**
Virus production	****	***

3.2 ViaFect™ Transfection Reagent

Application

Transfection of adherent cells, suspension cells, primary cells, and stem cells.

Description

ViaFect™ Transfection Reagent is a lipid-based reagent that can be directly used without the need of washing steps and/or medium exchange. It is suitable for transfection of adherent cells, suspension cells, primary cells, and stem cells. The protocol allows transfection at low cell density with minimal toxicity. Laborious optimization is no longer required.

Features

- High transfection efficiency at low toxicity
- Easy handling without medium exchange and washing of cells
- Simple protocol with minimal optimization
- Tested for a variety of cell lines

Reference

Koschut, D *et al.* (2016) Live cell imaging show hepatocyte growth factor-induced Met dimerization. Biochim. Biophys. Acta 1863, 1552-8.

Straka, E *et al.* (2016) Mercury toxicokinetics of the healthy human term placenta involve amino acid transporters and ABC transporters. Toxicology 340, 34-42.

Horita, H *et al.* (2016) Nuclear PTEN functions as an essential regulator of SRF-dependent transcription to control smooth muscle differentiation. Nat. Comm. 7, 10830.

Product Box

ViaFect™ Transfection Reagent

Cat.# E4981, E4982

Tab. 3.2 Comparison of ViaFect™ and Lipofectamine®

	ViaFect™		Lipofectamine® 2000	
Cell line	Transfection efficiency	Cell viability	Transfection efficiency	Cell viability
HEK293	❹	❹	❸	❶
HeLa	❹	❹	❸	❷
MCF7	❹	❹	❹	❸
U2OS	❹	❹	❶	❸
A549	❹	❹	❷	❶
LNCap	❹	❹	❶	❷
PC3	❹	❹	❶	❸
HCT116	❹	❹	❹	❷
HT-29	❹	❹	❹	❸
HepG2	❹	❸	❶	❶
HUH7	❹	❸	❸	❷
K562	❸	❹	❹	❹
Jurkat	❹	❹	❸	❹
C2C12	❹	❹	❶	❹
Raw264.7	❶	❹	❹	❷
NIH 3T3	❸	❹	❹	❸
COS7	❹	❹	❹	❸
CHO	❹	❹	❸	❶
H9C2	❹	❹	❷	❸
PC12	❹	❹	❹	❸
Average	**3.70**	**3.95**	**2.80**	**2.50**

Comparison of ViaFect™ and Lipofectamine® 2000 Reagents regarding transfection efficiency and cell viability

❶ low transfection efficiency/cell viability

❹ high transfection efficiency/cell viability

HEK293 cell were transfected with 50 ng (5 µL) or 100 ng (10 µL) of a luciferase reporter plasmid using either ViaFect™ or Lipofectamine® 2000 transfection reagent (reagent/DNA ratio 2:1, 2,5:1, 3:1, 4:1) in a 96-well plate. Luciferase expression and cell viability were determined after 24 hours using ONE-Glo™ + Tox Assays.

4 Overview of Luciferase Reporter Assays and Substrates

Promega's luciferase detection assays have been developed and optimized for various applications using mammalian cells. They all contain compatible substrate-buffer systems including cell lysis reagents for lytic assays.

Luciferase detection systems are mostly **homogenous assays**, which are based on the simple ***add-mix-measure*** format. In this format, the detection reagent is directly added to the cell culture medium. The incubation time between reagent addition and signal detection largely depends on the half-life of the luminescent signal. In case of ***flash-type assays*** the sample is directly measured upon addition of the detection reagent since the signal half-life is in the range of minutes. In contrast, ***glow-type assays*** (Glo™ Assays) exhibit a prolonged signal half-life – in some cases several hours – which is particulary beneficial for high-throughput applications. Flash-type assays usually possess a slightly higher sensitivity compared to corresponding glow-type assays. However, they are limited in terms of sample throughput and reproducibility. Therefore, Glo™ Assays are highly recommended when sample throughput and reproducibility are indispensable.

Classical reporter gene assays generally utilize lytic detection reagents. In lytic assays, luciferase activity is recorded at a single time point (endpoint measurement). They are available as **single assays** for activity measurements of single luciferases or as **dual assays** that allows for the detection of two luciferases per sample. By contrast, live-cell substrates are used for kinetic live-cell measurements.

Tab. 4.1 Overview of Promega's luciferase detection systems

	Product	Luciferase reporter gene	Glo/ Flash[1]	Assay format	Substrate	Signal half-life	Signal stability	Signal intensity	Cat.#
Single Reporter Systems (lytic; endpoint measurement)									
	Nano-Glo® Luciferase Assay System	**NanoLuc:** Nluc, secNluc[2]	Glo	Homogenous	Furimazine	≥ 2 h	4+	6+	N1110
	Luciferase Assay System	**Firefly:** luc, luc+, luc2	Flash	Requires cell lysate	Beetle luciferin	~ 5-10 min	1+	5+	E1483
	Bright-Glo™ Luciferase Assay System	**Firefly:** luc, luc+, luc2	Glo	Homogenous	Beetle luciferin	~ 30 min	2+	4+	E2610
	ONE-Glo™ Luciferase Assay System	**Firefly:** luc, luc+, luc2	Glo	Homogenous	5' - Fluoroluciferin	≥ 45 min	2+	3+	E6110
	ONE-Glo™ EX Luciferase Assay System	**Firefly:** luc, luc+, luc2	Glo	Homogenous	5' - Fluoroluciferin	≥ 2h	4+	2+	E8110
	Steady-Glo® Luciferase Assay System	**Firefly:** luc, luc+, luc2	Glo	Homogenous	Beetle luciferin	> 5 h	5+	1+	E2510
	Renilla Luciferase Assay System	**Renilla:** Rluc, hRluc	Flash[1]	Requires cell lysate	Coelenterazine	~ 5-10 min	1+	4+	E2810
	Renilla-Glo™ Luciferase Assay System	**Renilla:** Rluc, hRluc	Glo	Homogenous	Coelenterazine-h	> 60 min	3+	2+	E2710
Dual Reporter Systems (lytic; endpoint measurement)									
	Nano-Glo® Dual-Luciferase® Reporter Assay System	NanoLuc®/ Firefly	Glo	Homogenous	Furimazine/ Beetle luciferin	Nluc: ~ 2h, Fluc: ~ 2 h	4+	6+/3+	N1610
	Dual-Luciferase® Reporter Assay System	Firefly/ Renilla	Flash[1]	Requires cell lysate	Beetle luciferin/ Coelenterazine	FLuc: ~ 8 min, RLuc: ~ 8 min	1+	4+/4+	E1910
	Dual-Glo® Luciferase Assay System	Firefly/ Renilla	Glo	Homogenous	Beetle luciferin/ Coelenterazine	Fluc: ~ 2 h, Rluc: ~ 2 h	4+	2+/2+	E2920
Live Cell Substrates (kinetics; single point measurement)									
	Nano-Glo® Live Cell Assay System	NanoLuc®; NanoBiT®	Glo	Add-only	Furimazine	~ 2 h	4+	6+	N2011
	NanoBRET™ Nano-Glo® Detection System	NanoLuc®; NanoBRET™	Glo	Add-only	Furimazine	~ 2 h	4+	6+	N1661
	VivoGlo™ Luciferin, In Vivo Grade	Firefly luc, luc+, luc2	ND	Add-only	Potassium salt D-luciferin	ND	ND	ND	P1041
	Luciferin-EF™ Endotoxin-Free Luciferin Na	Firefly luc, luc+, luc2	ND	Add-only	Luciferin	ND	ND	ND	E6551
	ViviRen™ Live Cell Substrate	Renilla Rluc, hRluc	Flash[1]	Add-only	Modified Coelenterazine	8-15 min	1+	4+	E6491
	EnduRen™ Live Cell Substrate	Renilla Rluc, hRluc	Glo	Add-only	Modified Coelenterazine	> 24h	6+	2+	E6481

[1] Flash-type assays require the use of automated injectors
[2] Detection of secreted NanoLuc® (secNluc) in the supernatant enabling kinetic measurements.

Note:
Homogenous Assay requires only addition steps.

5 Single Reporter Assays for the Quantification of Luciferase Activity (endpoint; lytic)

Scheme of a single-reporter gene assay

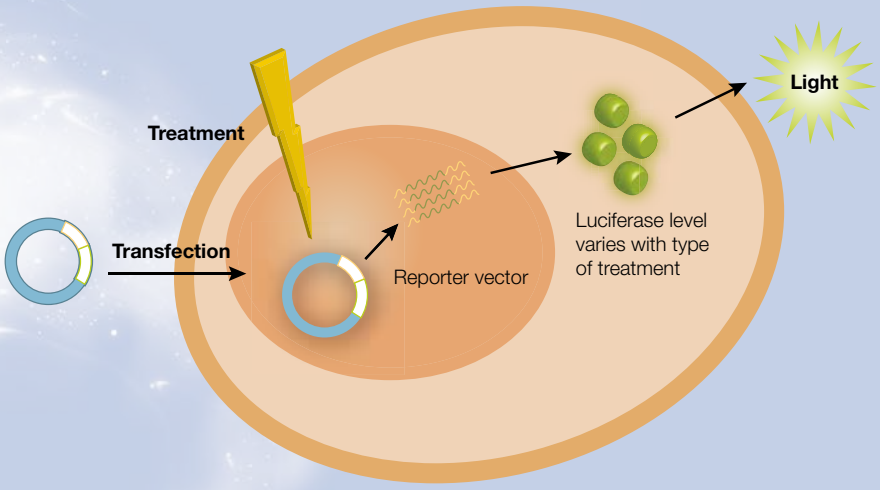


Fig. 5.1 Generalized procedure of a single-reporter assay

Single Reporter Assays for the Quantification of Luciferases

The activity of a single reporter luciferase can be determined by using a compatible substrate-buffer solution and the reagent for cell lysis. Generally, the workflow of a single-reporter assay starts with transfection of the reporter construct. Reporter activity is determined by adding single-luciferase assay reagent to cultured cells (Fig. 5.1). The various single-luciferase assays differ in initial signal intensity and signal stability (Fig. 5.2). The Nano-Glo® Luciferase Assay System for the detection of NanoLuc® luciferase activity is striking because of its very high signal intensity and enduring signal stability.

Comparison of various single-reporter assay systems

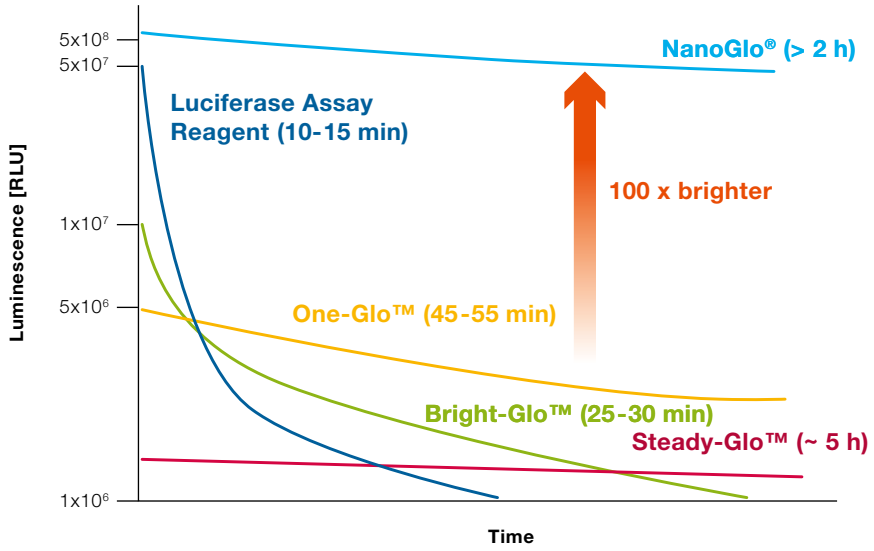


Fig. 5.2 Signal intensity and signal stability of various single assays systems detecting either firefly or NanoLuc® luciferase activities. Single assays systems for the detection of Renilla luciferase are not shown.

5.1 Single Reporter Assays for the Detection of NanoLuc® Luciferase

Nano-Glo® Luciferase Assay System

Highest sensitivity and stability at the same time

Application

Determination of NanoLuc® luciferase reporter activity in gene regulation, signal transduction or protein stability experiments. Applicable to HT screens.

Assay Description

The Nano-Glo® Luciferase Assay System is used for the determination of NanoLuc® luciferase activity. It is a homogeneous glow-type assay with long signal stability ($t_{1/2}$ = 120 minutes). The assay is linear over 6 orders of magnitude and extremely sensitive due to its high signal intensity. The assay reagent contains the luciferase substrate furimazine and an optimized buffer system with cell-lytic components. The activity of NanoLuc® is determined by direct addition of assay reagent onto cultured cells. For the detection of secreted NanoLuc® (secNluc), the assay reagent is added to the supernatant which has previously been transferred to a separate assay plate.

Assay Features

Luciferase	NanoLuc® (Nluc), secreted NanoLuc® (secNluc) with IL-6 leader sequence
Assay type	Single assay, glow-type
Implementation	Homogeneous, 1-step assay
Substrate	Furimazine
Signal half-life	≥ 2 h
Signal linearity	5-6 logs
Sample material	Mammalian cells, medium (secNLuc)
HTS capability	Tested in 96-, 384- and 1536-well

References

Hall, MP *et.al.* (2012) Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. ACS Chemical Biology 7(11), 1848-572012.

Hsu, CW *et al.* (2016) Identification of approved and investigational drugs that inhibit hypoxia-inducible factor-1 signaling. Oncotarget 7, 8172-8183.

Product Box

Nano-Glo® Luciferase Assay System
Cat.# N1110, N1120, N1130, N1150

High sensitivity of Nano-Glo® Assay allows to work at physiologically relevant expression conditions

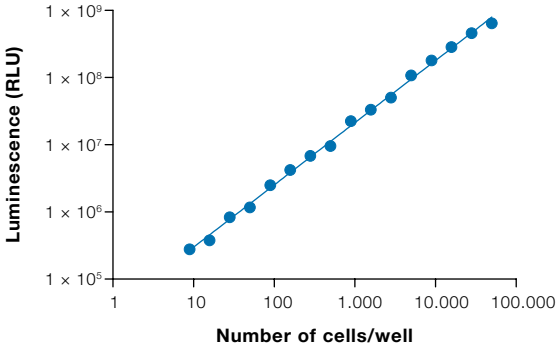


Fig. 5.3 Detection of NanoLuc® expression in transiently transfected cells (10-100,000 cells). HEK293 cells were transiently transfected with the vector pNL1.1 [CMV] and serially diluted with complete growth medium in a 96-well plate. Luminescence was recorded 10 min after adding the Nano-Glo® Luciferase Assay Reagent.

NanoLuc® allows detection of weak signal responses

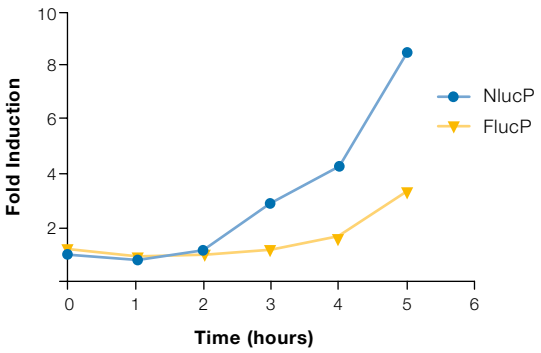


Fig. 5.4 Comparison of the heat-shock response as measured with NanoLuc® and firefly luciferase. Cells were transfected with the heat-shock response vectors pNL[NlucP/HSE/Hygro] or pGL4.41[luc2P/HSE/Hygro]. The heat-shock response was induced by treatment with 17-AAG (17-allylamino-17-demethoxygeldanamycin). This leads to the activation of transcription factor HSF1, which in turn activates transcription of downstream genes by binding to heat-shock DNA sequence elements (HSE). Cells transfected with the NlucP reporter construct yielded an enhanced response.

5.2 Single Reporter Assays for the Detection of Firefly Luciferase

Luciferase Assay System

Most sensitive firefly assay (non-homogeneous; flash-type)

Application

Determination of firefly luciferase reporter activity. Investigation of gene expression, signal transduction and mRNA processing.

Assay Description

The Luciferase Assay System is an extremely sensitive reagent for rapid detection of the firefly luciferase activity. The assay is characterized by a large linear range over 8 orders of magnitude. Moreover, it confers extremely high sensitivity, detecting as little as 10^{-20} mol luciferase. The Luciferase Assay System is a flash-type assay with a very high initial signal intensity. The signal half-life is around 5–10 minutes. Therefore, the use of automated injectors for the addition of assay reagent is highly recommended. The assay is compatible with various lysis buffers. Cell lysis is conducted prior to substrate addition. For a description of homogenous "add-read" glow-type firefly luciferase assays, like Bright-Glo™, Steady-Glo®, ONE-Glo™ or ONE-Glo™ EX, refer to pages 50–53.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Assay type	Single assay, flash-type
Implementation	Non-homogeneous, separate cell lysis is required
Substrate	Beetle luciferin
Signal half-life	~ 5 -10 min
Signal linearity	8 logs
Sample material	Cell lysates: plants, mammalian cells, bacteria
HTS capability	Limited; tested in 96-well plates
Injectors	Recommended

References

Sun, C *et al.* (2014) Stable, high-level expression of reporter proteins from improved alphavirus expression vectors to track replication and dissemination during encephalitic and arthritogenic disease. J. Virol. 88, 2034–2046.

Jiang, C *et al.* (2014) A bioluminescence assay for DNA methyltransferase activity based on methylation-resistant cleavage. Anal. Biochem. 423(2), 224-228.

Fuentealba, RA *et al.* (2012) An aggregation sensing reporter identifies leflunomide and teriflunomide as polyglutamine aggregate inhibitors. Hum. Mol. Genet. 21(3), 664-680.

High sensitivity and broad linear range

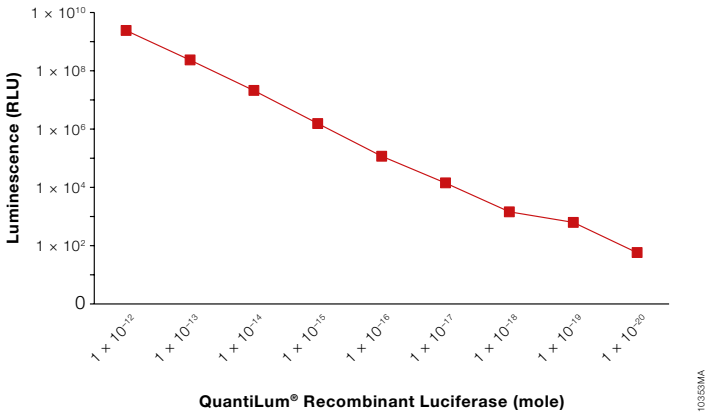


Fig. 5.5 Dynamic range and high sensitivity of the Luciferase Assay System as measured with a GloMax® luminometer. A dilution series from 1 × 10^{-10} mol to 1 × 10^{-21} mol of a recombinant firefly luciferase (QuantiLum® Recombinant Luciferase Cat.# E1701) was prepared and luminescence was detected after adding the Luciferase Assay Reagent. The dynamic range spans 8 orders of magnitude.

Workflow of the Luciferase Assay System

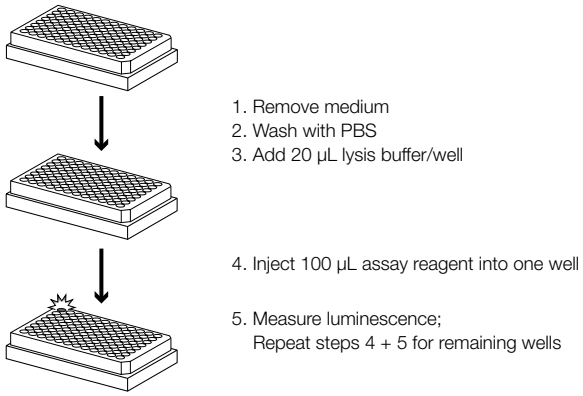


Fig. 5.6 The flash-type Luciferase Assay System is a non-homogeneous assay that requires the cells to be lysed prior to the addition of the assay reagent. For the latter, use of injectors integrated into the luminometer is highly recommended.

Product Box

Luciferase Assay System
Cat.# E1483, E1500, E1501, E1531, E4530, E4550

Bright-Glo™ Luciferase Assay System

Most sensitive homogeneous firefly glow-type assay

Application

Determination of firefly luciferase reporter activity. Investigation of gene expression, signal transduction, mRNA processing and compound screening. Applicable for HT screens in the continuous processing mode.

Assay Description

The Bright-Glo™ Luciferase Assay System is a homogeneous 1-step assay for the quantification of firefly luciferase activity in mammalian cells, which is easily adopted for high-throughput applications. Amongst the available glow-type firefly assays it is the most sensitive (<10^{-19} mol luciferase corresponding to 5 fg) with a signal stability of around 30 minutes. The assay is compatible with various types of cell culture media and fetal calf serum (FCS). Cell lysis is completed within 2 minutes after the addition of assay reagent.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Assay type	Single assay, glow-type
Implementation	Homogeneous, 1-step assay
Substrate	Beetle luciferin
Signal half-life	~ 30 min
Sensitivity	< 10^{-19} mol luciferase
Signal linearity	> 7 logs
Sample matierial	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well
Robust	Compatible with various media and media supplements
Cell lysis	Completed 2 min after the addition of assay reagent

References

Xia, M *et al.* (2009) Identification of compounds that potentiate CREB signaling as possible enhancers of long-term memory. Proc.Natl. Acad. Sci. USA 106, 2412-7.

Bridges, JP *et al.* (2005) Expression of a human surfactant protein C mutation associated with interstitial lung disease disrupts lung development in transgenic mice. J. Biol. Chem. 278, 52739-46.

Signal intensities and signal half-lifes of glow-type firefly substrates

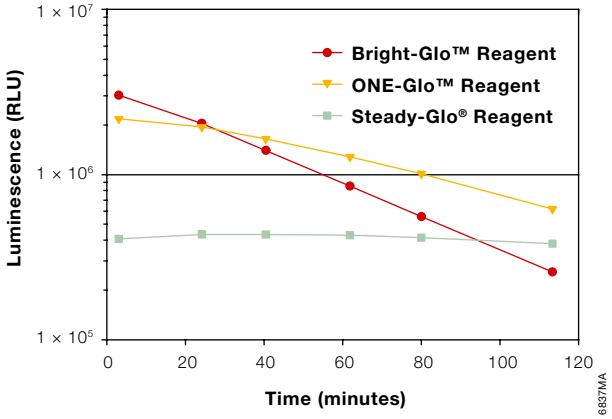


Fig. 5.7 Bright-Glo™ shows the highest luminescence signal up to 20 min after substrate addition.

Application of Bright-Glo™ for the continuous processing in HT screens

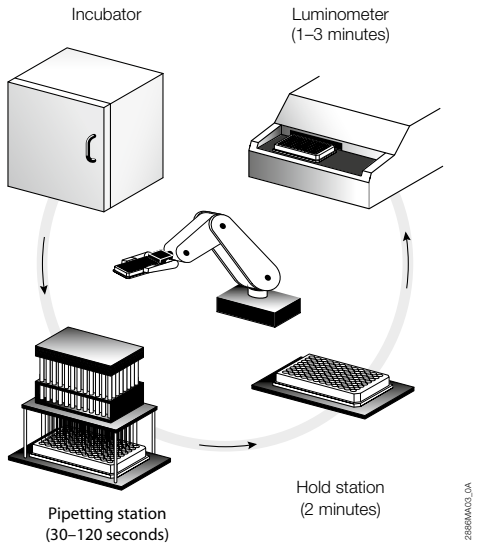


Fig. 5.8 Assay plates are continuously processed for screens with Bright-Glo™.

Product Box

Bright-Glo™ Luciferase Assay System
Cat.# E2610, E2620, E2650

ONE-Glo™ Luciferase Assay System

Application

Determination of firefly luciferase activity in studies on gene expression, signal transduction, and mRNA processing. Applicable to HT screens.

Assay Description

The ONE-Glo™ Luciferase Assay System is based on a modified firefly luciferase substrate conferring an optimized signal intensity and prolonged signal stability when compared to the Bright-Glo™ Luciferase Assay System. Additionally, it exhibits improved tolerance to cell culture media supplements and compounds. The ONE-Glo™ Luciferase Assay System is ideally suited to detect firefly expression within mammalian cells and it is easily adaptable to HT screening and ultra-HT screening approaches.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Assay type	Single assay, glow-type
Implementation	Homogeneous, 1-step assay
Substrate	5'-Fluoroluciferin
Signal half-life	≥ 45 min
Signal linearity	7 logs
Sample material	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well
Reagent stability	Increased storage stability at RT or 4 °C
Smell	Odorless in comparison to Steady-Glo® or Bright-Glo™

References

Zhou, Q *et al.* (2016) Topoisomerase IIα mediates TCF-dependent epithelial-mesenchymal transition in colon cancer. Oncogene. 35(38):4990-9.

Cai, Y *et al.* (2014) DNA transposition by protein transduction of the *piggyBac* transposase from lentiviral Gag precursors. Nucl. Acids Res. 42, e28.

Product Box

ONE-Glo™ Luciferase Assay System
Cat.# E6110, E6120, E6130

High signal linearity

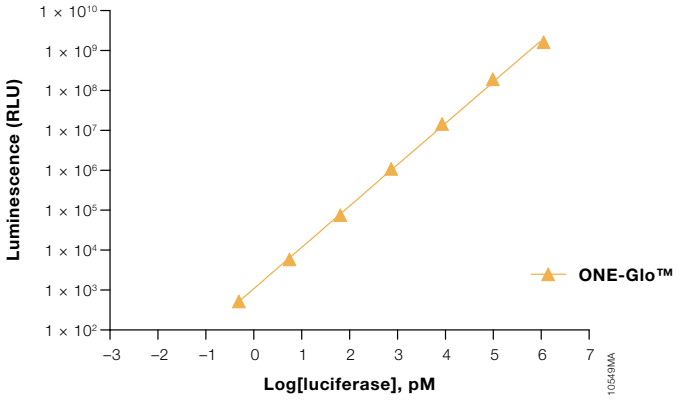


Fig. 5.9 The ONE-Glo™ Luciferase Assay System yields robust signals over a broad concentration range (~ 7 orders of magnitude).

Improved reagent stability

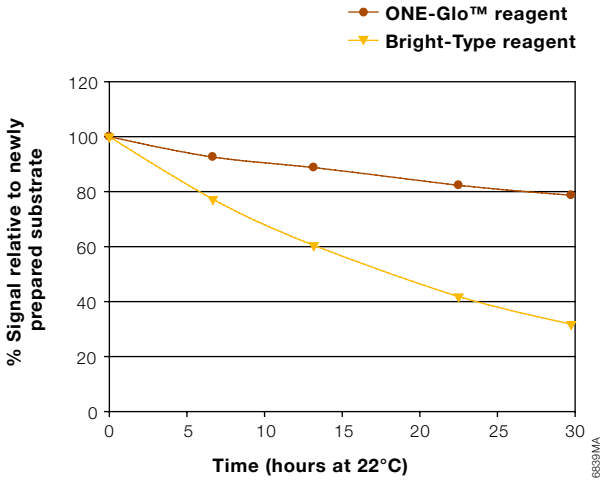


Fig. 5.10 The reagent stability of ONE-Glo™ is considerably higher than that of bright-type reagents from other manufacturers.

ONE-Glo™ EX Luciferase Assay System

Application

Determination of firefly luciferase reporter activity in studies of gene expression, signal transduction, and mRNA processing. Applicable to HT screens.

Assay Description

The ONE-Glo™ EX Assay System utilizes a novel assay chemistry with improved signal and reagent stability compared to the ONE-Glo™ Reagent. With a signal half-life of 2 hours the reagent is ideally suited for HT or ultra-HT screenings. The prolonged stability of the reconstituted reagent facilitates decreased data variability and diminished reagent consumption.

Special Features of the ONE-Glo™ EX Reagent

- Prolonged stability of the reconstituted reagent
- 1.5-fold extended signal stability (ONE-Glo™ EX $t_{1/2}$ = 120 min vs ONE-Glo™ $t_{1/2}$ = 45 min)
- Robust against quenching effects of luminescence by phenol-red
- Odor-causing thiol compounds are not included

References

Gromadzka, AM et al. (2016) A short conserved motif in ALYREF directs cap- and EJC-dependent assembly of export complexes on spliced mRNAs. Nucl. Acids Res. 44, 2348-2361.

Kobayashi, EH *et al.* (2016) Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat. Comm. 7, 11624.

Product Box

ONE-Glo™ EX Luciferase Assay System
Cat.# E8110, E8120, E8130, E8150

Increased stability of the ONE-Glo™ EX Reagent

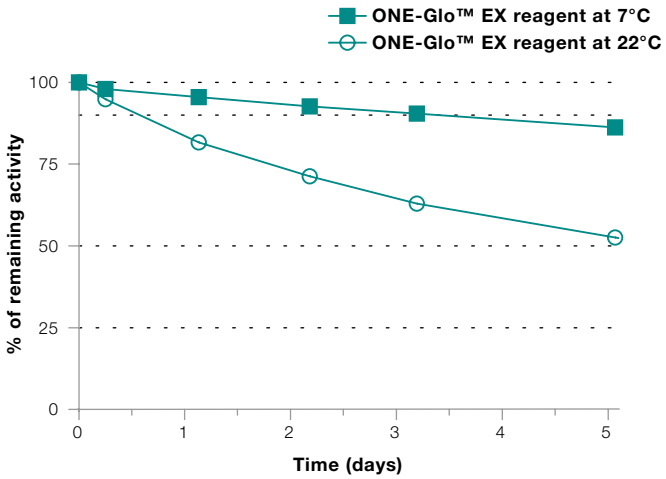


Fig. 5.11 The reconstituted ONE-Glo™ EX Reagent exhibits prolonged stability as shown for 22°C and 7°C.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Assay type	Single assay, glow-type
Implemetation	Homogeneous, 1-step assay
Substrate	5'-fluoroluciferin
Signal half-life	≥ 2 h
Reagent stability	Improved storage stability at RT
Sample material	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well

Steady-Glo® Luciferase Assay System

Application

Determination of firefly luciferase reporter activity in studies on gene expression, signal transduction, and mRNA processing. Applicable to HT screens.

Assay Description

The Steady-Glo® Luciferase Assay System is a firefly luciferase detection reagent with an extremely long signal stability ($t_{1/2}$ ~ 5 hours). This enables highly reproducible processing of more than one thousand samples per hour.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Assay type	Single assay, glow-type
Implementation	Homogeneous, 1-step assay
Substrate	Beetle luciferin
Signal half-life	> 5 h
Sample material	Mammalian cells
HTS capability	Tested in 96- and 384-well

References

Thorne, CA *et al.* (2011) A biochemical screen for identification of small-molecule regulators of the wnt pathway using *Xenopus* egg extracts. J. Biomol. Scr. 16(9), 995–1006.

Neklesa, TA *et al.* (2011) Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. Nat Chem Biol. 7(8):538-43.

Rousseau, J *et al.* (2010) Advantages of bioluminescence imaging to follow siRNA or chemotherapeutic treatments in osteosarcoma preclinical models. Cancer Gene Ther. 17(6), 387–97.

Product Box

Steady-Glo® Luciferase Assay System
Cat.# E2510, E2520, E2550

Detection of firefly expression using Steady-Glo®

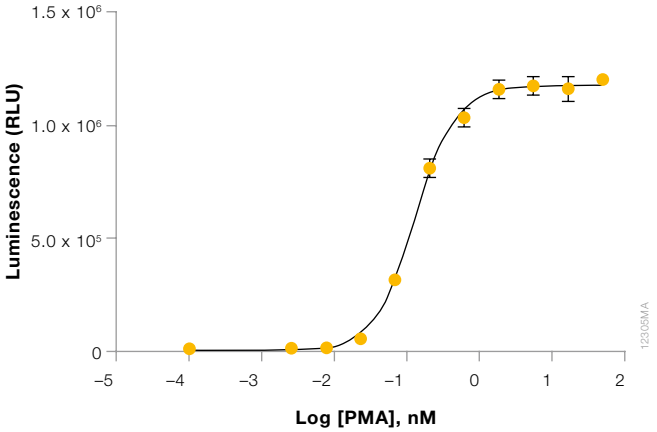


Fig. 5.12 Stably transfected HEK293 cells in a 96-well plate (vector pGL4.30[luc2P/NFAT-RE/Hygro]) were treated with PMA/Ionomycin. Induction of firefly luciferase expression in response to different concentrations of PMA was determined using the Steady-Glo® Reagent and a GloMax® Discover luminometer.

Batch application of Steady-Glo® in HT screens

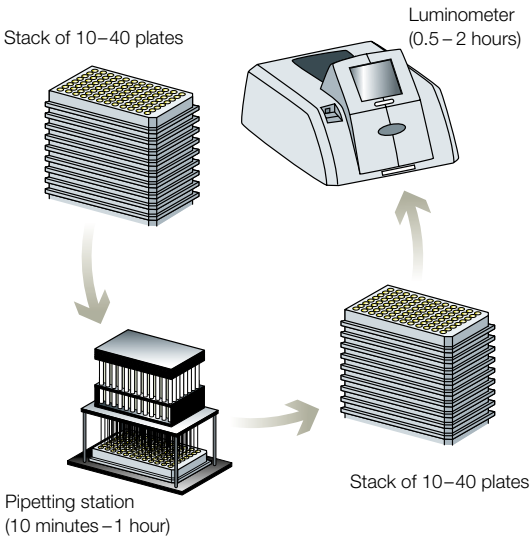


Fig. 5.13 The Steady-Glo® Reagent enables the measurement of luciferase activity in batch mode. Addition of Steady-Glo® Reagent to all samples occurs prior to the determination of luminescence.

5.3 Single Reporter Assays for the Detection of Renilla Luciferase

Renilla Luciferase Assay System

Application

Determination of Renilla luciferase reporter activity.

Assay Description

The Renilla Luciferase Assay System comprises an extremely sensitive detection reagent to measure Renilla luciferase activity. This flash-type assay yields high signal intensity and linearity over a wide concentration range (7 orders of magnitude). The Renilla Luciferase Assay Reagent significantly reduces colenterazine-associated autoluminescence that is frequently observed with other Renilla reagents. This reagent enables activity measurements of wildtype and synthetic Renilla luciferase in expression studies.

Assay Features

Luciferase	Renilla: Rluc, hRluc
Assay type	Single assay, flash-type
Implementation	Inhomogeneous, 2-step assay
Substrate	Coelenterazine
Signal half-life	~ 3 min
Signal linearity	7 logs
Sample material	Mammalian cells
HTS capability	Limited; tested in 96-well plates
Injectors	Recommended

References

Walter, RW *et al.* (2010) Poly(A)-binding protein modulates mRNA susceptibility to cap-dependent miRNA-mediated repression. RNA 16, 239–50.

Mie, M *et al.* (2008) Selection of mRNA 5′-untranslated region sequence with high translation efficiency through ribosome display. Biochem. Biophys. Res. Commun. 373, 48–52.

Fan, F *et al.* (2007) Bioluminescent assays for high-throughput screening. Assay Drug Dev. Technol. 5, 127–136.

Product Box

Renilla Luciferase Assay System
Cat.# E2810, E2820

High signal intensity and linearity extending over a 7 log-range

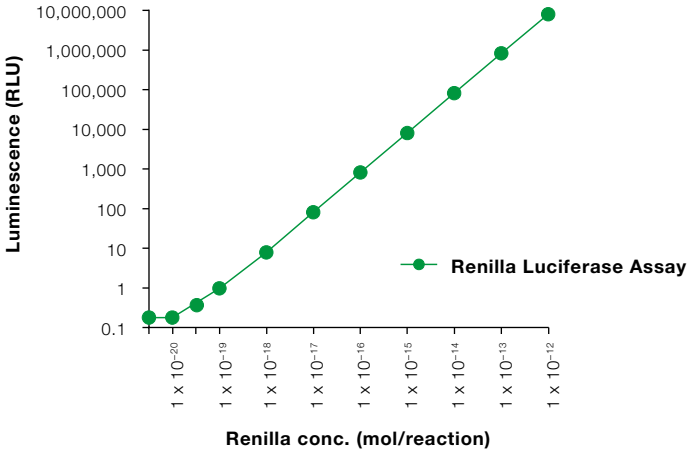


Fig. 5.14 The activity of purified Renilla luciferase was determined using the Renilla Luciferase Assay Reagent.

Workflow of the Renilla Luciferase Assay System

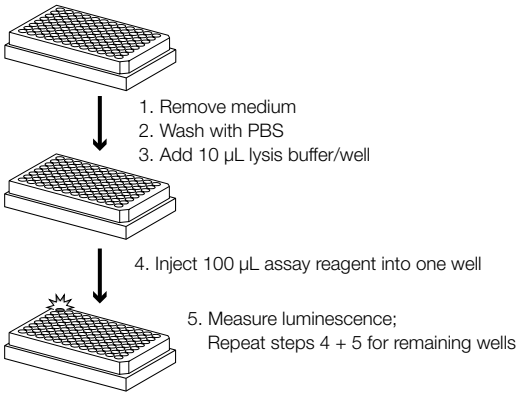


Fig. 5.15 The flash-type Renilla Luciferase Assay System is a non-homogeneous assay that requires the cells to be lysed prior to the addition of the assay reagent. It is recommended to perform the step of reagent addition with automated injectors integrated into the luminometer.

Renilla-Glo™ Luciferase Assay System

Application

Determination of Renilla luciferase reporter activity.

Assay Description

The Renilla-Glo™ Luciferase Assay System follows the convienent add and read principle. When added to the cells it yields a stable luminescent signal with a half-life greater than 1 hour. Renilla-Glo™ is particularly suited when working with luminometers lacking injectors and can be easily scaled up to larger plate formats (384- and 1563-wells). The improved buffer composition substantially reduces autoluminescence that is commonly observed with other coelenterazine-based assays.

Assay Features

Luciferase	Renilla: Rluc, hRluc
Assay type	Single assay, glow-type
Implementation	Homogeneous, 1-step assay
Substrate	Coelenterazine-h
Signal half-life	> 60 min
Signal linearity	7 logs
Sample material	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well

References

Lucas-Hourani, M *et al.* (2013) A phenotypic assay to identify Chikungunya virus inhibitors targeting the nonstructural protein nsP2. J Biomol Screen. 18(2):172-9.

Williams, V *et al.* (2012) Large hepatitis delta antigen activates STAT-3 and NF-κB via oxidative stress. J Viral Hepat. 19(10):744-53.

Product Box

Renilla-Glo™ Luciferase Assay System
Cat.# E2710, E2720, E2750

High signal intensity and linearity over 7 orders of magnitude

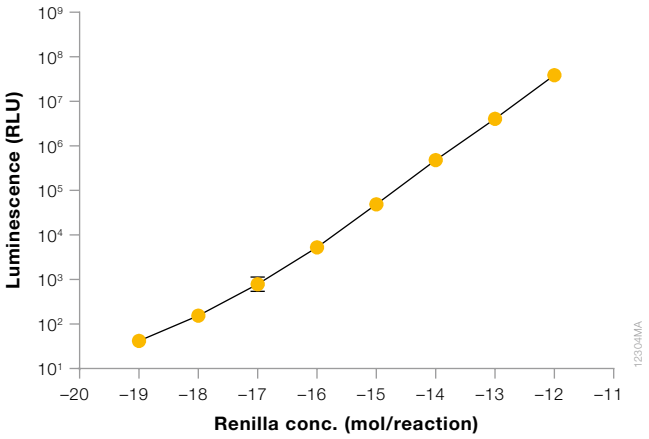


Fig. 5.16 Renilla luciferase activity was determined using the Renilla-Glo™ Luciferase Assay System in a 96-well plate and a GloMax® Discover luminometer.

Glow-type kinetics of the Renilla-Glo® Assay

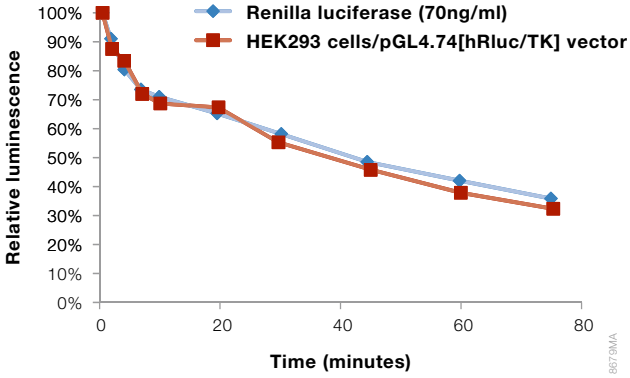


Fig. 5.17 Luminescence kinetics determined with purified Renilla luciferase and Renilla-expressing HEK293 cells.

6 Dual Assays for the Quantification of Two Luciferases in a Single Sample (endpoint, lytic)

Dual assay format: Measurement of two luciferase reporters in a single sample

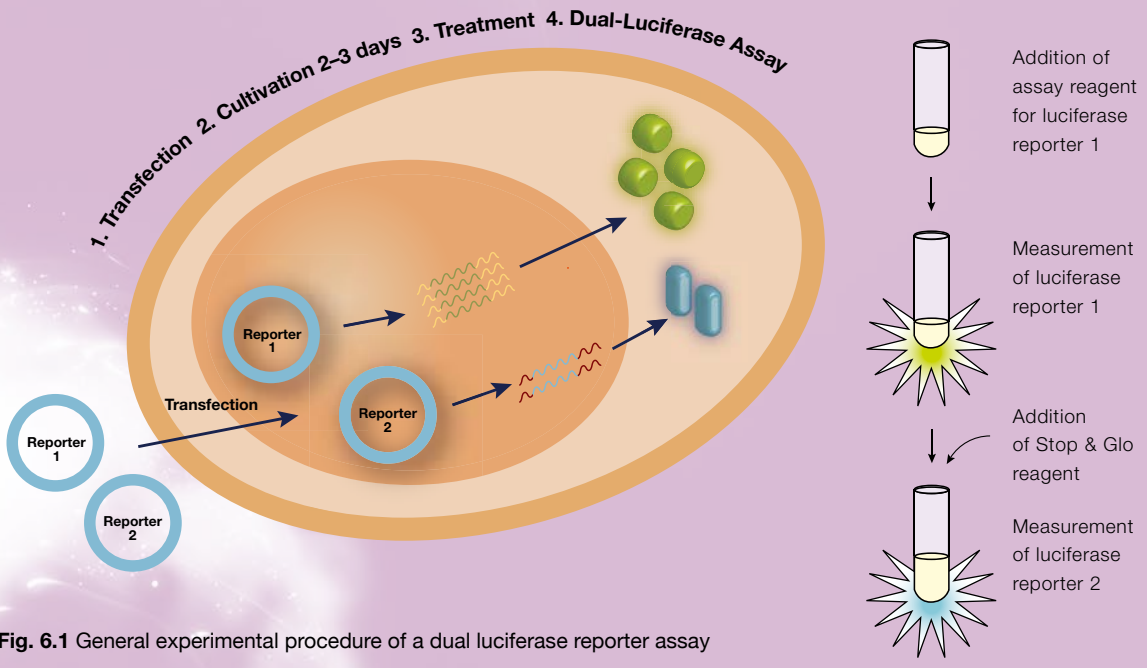


Fig. 6.1 General experimental procedure of a dual luciferase reporter assay

Overview of Dual Assays for the Quantification of Two Luciferases in a Single Sample

In a dual-luciferase assay the activity of two different luciferase reporters is measured in a single sample. The assay chemistry of dual assay reagents allows for substrate addition and determination of luciferase activity in a consecutive manner. Different luciferase reporters can be combined as shown in Table 6.1.

Applications of dual reporter assays

- **Normalization** of reporter data obtained with transiently transfected cells, recommended for promoter studies (chapter 2.1), analyses of signal transduction (chapter 2.2) and analyses of RNA interference (chapter 2.3).
- Measuring **two cellular responses** in a single sample such as protein stability and genetic pathway reporter activity (chapter 6.1).
- **Compound HT screening** with coincidence reporter vectors (chapter 2.3).

Tab. 6.1 Dual assay formats and possible luciferase reporter combinations.

	Dual-Luciferase® Reporter (DLR™) Assay	Dual-Glo® Assay	Nano-Glo® Dual-Luciferase® Reporter (NanoDLR™) Assay
Luciferases	Firefly + Renilla	Firefly + Renilla	Firefly + NanoLuc®
Format	Non-homogeneous	Homogeneous	Homogeneous (also compatible with cell lysates)
Sample processing	Bench-scale	Bench to batch	Bench to batch
Number of steps	5	2 (add-only)	2 (add-only)
Sensitivity	high	medium	high
Firefly signal-$t_{1/2}$	~ 9 minutes	~ 2 hours	~ 2 hours
Renilla signal-$t_{1/2}$	~ 2 minutes	~ 2 hours	-
NanoLuc® signal-$t_{1/2}$	-	-	~ 2 hours
Z' factor	medium	high	high
Time required for cell lysis	~ 15 minutes	~ 10 minutes	~ 3 minutes

Normalization with an internal control-reporter using dual luciferase assays

Normalization to an internal control reporter is typically the best method to control for the variables introduced in a transfection-based experiment. This method uses a constitutively expressed control reporter vector that is co-transfected with the experimental reporter vector. The luminescence of both are measured sequentially using a dual- reporter assay, and a normalized ratio is obtained for each well by calculating "Experimental Reporter Activity/Control Reporter Activity". This method accounts for well-to-well variability caused by differences in transfection efficiency, cell number, number of viable cells or edge effects caused by the position on the plate. Detailed information about data normalization can be obtained from:
https://www.promega.de/resources/pubhub/designing-a-bioluminescent-reporter-assay-normalization/.

6.1 Dual Assays Combining NanoLuc® and Firefly Luciferases

Nano-Glo® Dual-Luciferase® Reporter Assay System

Application

Detection of firefly and NanoLuc® luciferase reporter activity in a single sample.

Assay Description

The Nano-Glo® Dual-Luciferase® Reporter Assay System (NanoDLR™) allows for the most sensitive detection of firefly and NanoLuc® luciferase activities in a single sample. The homogeneous add-only format and the long signal half-life of 2 hours qualify this assay for HT screens. The NanoDLR™ contains two reagents: (1) the ONE-Glo™ EX Luciferase Assay Reagent for determining firefly activity, and (2) the NanoDLR™ Stop & Glo® Reagent for quenching firefly luminescence and measuring NanoLuc® activity. Signal separation is excellent since the firefly signal is quenched by a factor of more than 1 x 10^6 through the addition of the Stop & Glo® Reagent (Fig. 6.4). Utilization of the extraordinarily bright NanoLuc® luciferase confers greater sensitivity when compared to Renilla luciferase that is part of the Dual-Glo® or DLR™ assays. NanoLuc® can be used as either, experimental and control reporter.

Assay features

Luciferases	NanoLuc®, Firefly (luc, luc+, luc2)
Assay type	Dual assay, glow-type
Implementation	Homogeneous, 2x addition steps
Substrate	Furimazine / 5'-fluoroluciferin
Signal half-life	~ 2 h
Sample material	Mammalian cells, cell lysates
HTS capability	Tested in 96-, 384- and 1536-well
Reagent stability	Increased storage stability

References

Hasson, SA *et al.* (2015) Chemogenomic profiling of endogenous PARK2 expression using a genome edited coincidence reporter. ACS Chem. Biol. 10(5):1188-97.

Ho, P *et al.* (2013) Reporter enzyme inhibitor study to aid assembly of orthogonal reporter gene assays. ACS Chem. Biol. 8, 1009–17.

Hall, M P *et al.* (2012) Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. ACS Chem. Biol. 7, 1848-57.

Workflow of the NanoDLR™ Assay

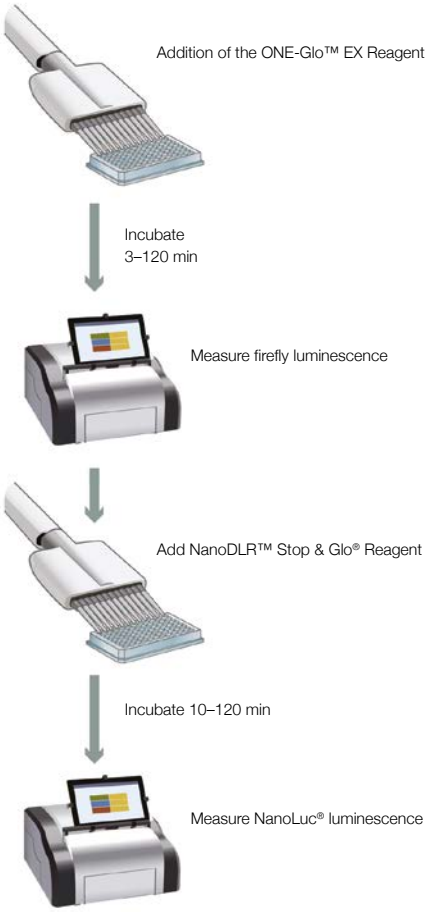


Fig. 6.2 In the NanoDLR™ Assay protocol, firefly luciferase activity is measured first using the ONE-Glo™ EX Luciferase Assay Reagent. Optionally cell lysates can be generated in advance using the Passive Lysis Buffer (Cat.# E1941). The ONE-Glo™ EX Reagent contains lytic components as well as the firefly substrate. After measuring the firefly luciferase activity the NanoDLR™ Stop & Glo® Reagent is added to quench the firefly signal and to provide the substrate for NanoLuc® luciferase.

Product Box

Nano-Glo® Dual-Luciferase® Assay System
Cat.# N1610, N1620, N1630, N1650

Application of NanoDLR™ to detect TNFα-mediated activation of NF-kB

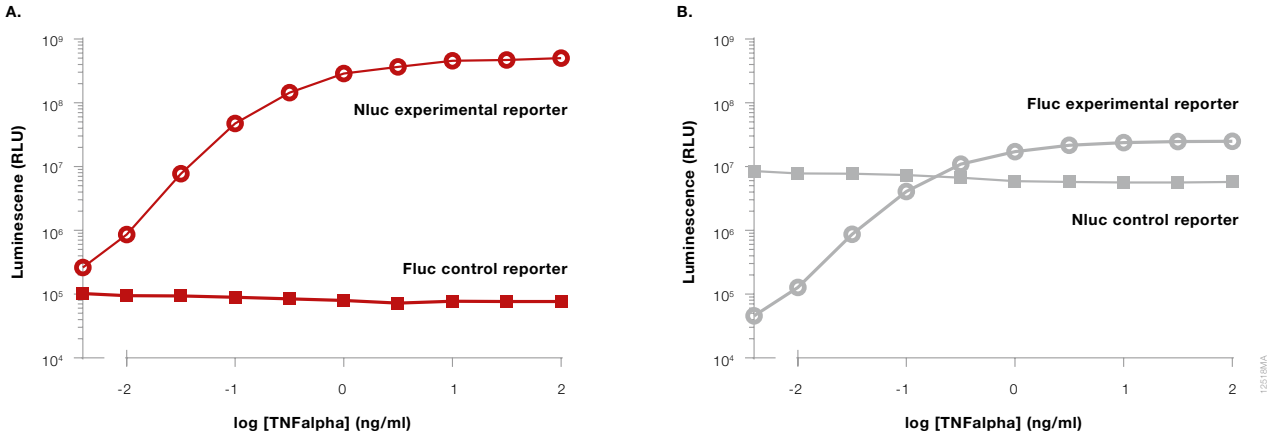


Fig. 6.3 Application of the NanoLuc® luciferase as **(A)** experimental reporter and as **(B)** control reporter. HEK293 cells were transfected with the following vectors: **(A)** pNL3.2.NF-kB-RE[NlucP/NF-kB-RE/Hygro] and pGL4.54[luc2/TK]; **(B)** pGL4.32[luc2P/NF-kB-RE/Hygro] and pNL1.1.TK[Nluc/TK]. The experimental/control vectors were transfected at a ratio of 10:1. Cells were stimulated with TNFα for at least 4 hours until luciferase activities were determined with the NanoDLR™ Assay.

The Fluc signal is efficiently quenched by the NanoDLR™ Stop & Glo® Reagent

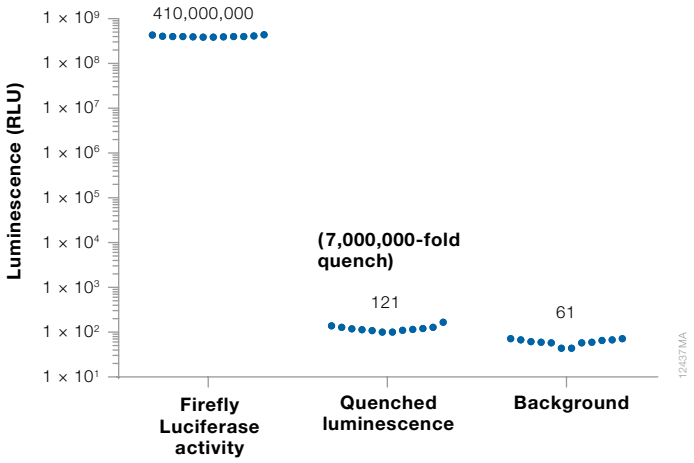


Fig. 6.4 In NanoDLR™ assays the firefly luciferase signal is quenched by a factor of > 1 x 10^6. The potent inhibition of firefly luciferase (Fluc) provides excellent signal separation, allowing accurate measurement of even low amounts of NanoLuc® luciferase (Nluc), even in the presence of high Fluc activity.

Detection of two cellular signal responses in a single sample

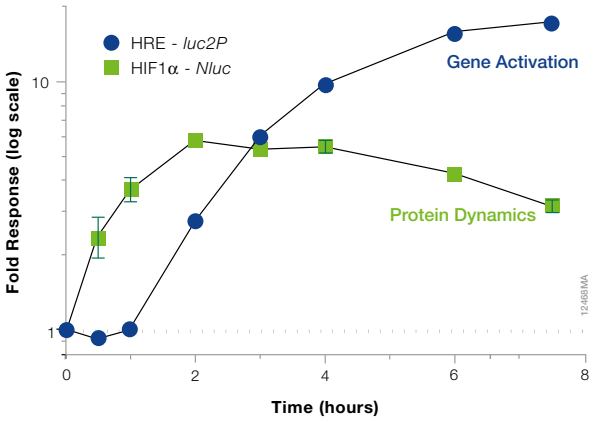


Fig. 6.5 Simultaneous determination of HIF1α transcription factor stabilization and HIF1α-meditated activation of transcription via binding to hypoxia response elements (HRE) in a single sample. HEK293 cells were transiently transfected with the protein stability sensor fusion construct pNLF1-HIF1A[CMV/neo] and the genetic reporter construct pGL4.42[luc2P/HRE/Hygro]. Hypoxia was induced 18 hours post-transfection by treatment with phenanthroline. Reporter activities were monitored using the NanoDLR™ Assay System at indicated time points.

6.2 Dual Assays Combining Firefly and Renilla Luciferases

Dual-Luciferase® Reporter Assay System

Application

Detection of firefly and Renilla luciferase reporter activity in a single sample.

Assay Description

The Dual-Luciferase® Reporter (DLR™) Assay System offers an efficient means to perform dual-reporter assays. With this assay, the activities of firefly and Renilla luciferase can be sequentially determined from a single sample. First, the activity of firefly luciferase is measured using the Luciferase Assay Reagent II (LAR II). Secondly, the Stop & Glo® Reagent is added to quench the firefly reaction and to initiate luminescence by Renilla luciferase.

Assay Features

Luciferases	Firefly, Renilla
Assay type	Dual assay, flash-type
Implementation	Non-homogeneous, 5 steps
Substrate	Beetle luciferin/coelenterazine
Signal half-life	FLuc: ~ 9 min, RLuc: ~ 2 min
Signal linearity	7 logs
Sample material	Cell lysates
HTS capability	Limited; tested in 96-well plates
Injectors	Prerequisite

Product Box

Dual-Luciferase® Reporter Assay System
Cat.# E1910, E1960, E1980

Linear range of the DLR™ Assay

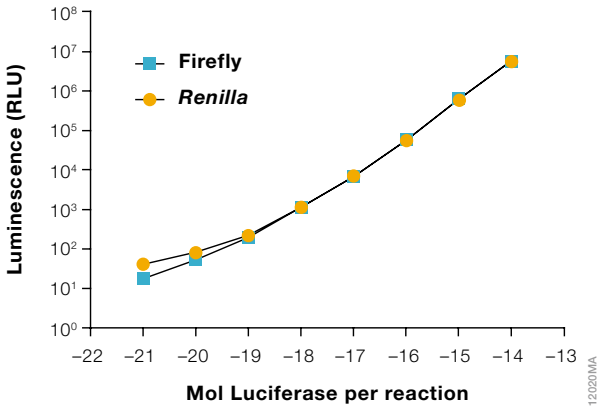


Fig. 6.6 The DLR™ Assay was performed with a mixture of purified firefly and Renilla luciferases using the Passive Lysis Buffer. The linear range of firefly and Renilla luciferase spans a concentration range of 7 orders of magnitude with a detection limit of ~ 10^{-21} moles luciferase per reaction.

References

Cheng, F *et al.* (2016) CSIRT1 promotes epithelial-mesenchymal transition and metastasis in colorectal cancer by regulating Fra-1 expression. Cancer Lett. 375, 274-83.

Im, JY *et al.* (2016) DNA damage-induced apoptosis suppressor (DDIAS), a novel target of NFATc1, is associated with cisplatin resistance in lung cancer. Biochim. Biophys. Acta 1863, 40–9.

Hayashi, M *et al.* (2016) Interaction of the hepatitis B virus X protein with the lysine methyltransferase SET and MYND domain-containing 3 induces activator protein 1 activation. *Microbiol Immunol.* 60, 17-25.

Satow, R *et al.* (2016) Phospoholipase Cδ1 induces E-cadherin expression and suppresses malignancy in colorectal cancer cells. Proc. Natl. Acad. Sci. USA 111, 13505–13510.

Dual-Glo® Luciferase Assay System

Application

Detection of firefly and Renilla luciferase reporter activity in a single sample.

Assay Description

The Dual-Glo® Luciferase Assay System is a homogeneous add-only assay for detecting firefly and Renilla luciferase activities in a single sample. The reagent yields a stable luminescence signal ($t_{1/2}$ = 2 h) and hence enables batch-mode measurements as applied in HTS. The Dual-Glo® Luciferase Assay System is composed of two reagents: (1) Dual-Glo® Luciferase Reagent and (2) Dual-Glo® Stop & Glo® Reagent. These reagents are sequentially added to the cells followed by the detection of luminescence. This assay is very robust, with high Z' values and compatible with common serum-supplemented cell culture media.

Assay Features

Luciferases	Firefly, Renilla
Assay type	Dual assay, glow-type
Implementation	Homogeneous; 2 addition steps
Substrate	Beetle luciferin/coelenterazine
Signal half-life	~ 2 h
Signal linearity	6 logs
Sample material	Mammalian cells
HTS capability	Tested in 96- and 384-well

Product Box

Dual-Glo® Luciferase Assay System
Cat.# E2920, E2940, E2980

Signal intensity and linearity of the Dual-Glo® Assay

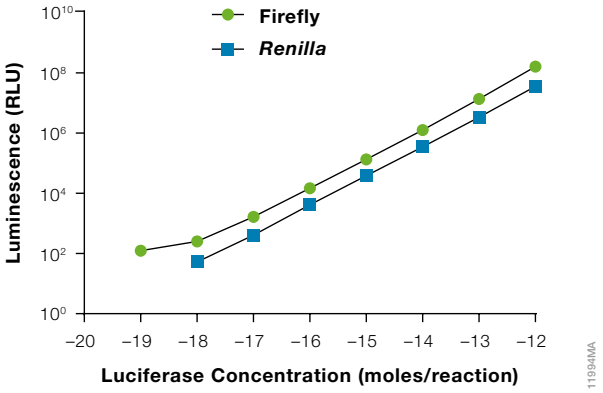


Fig. 6.7 The Dual-Glo® Assay was performed with a mixture of purified firefly and Renilla luciferases. The linear concentration range of both luciferases spans 6 orders of magnitude with a detection limit of ~ 10^{-18} mol luciferase per reaction.

References

Denicola, GM *et al.* (2015) NRF2 regulates serine biosynthesis in non-small cell lung cancer. Nat. Genet. 47, 1475–81.

Merhi, A *et al.* (2015) Wnt/β-catenin signaling regulates the expression of the ammonium permease gene RHBG in human cancer cells. PLos ONE 10(6):e0128683.

Muruoka, N *et al.* (2014) MiR-133 promotes cardiac reprogramming by directly repressing Snai1 and silencing fibroblast signatures. EMBO J. 33, 1565-1581.

7 Live-Cell Substrates for Dynamic Real-Time Measurements and Imaging

7.1 Live-Cell Substrates for the Detection of NanoLuc® Luciferase

Nano-Glo® Live Cell Assay System

Application

Kinetic measurements of NanoBiT® or NanoLuc® luminescence inside living cells.

Assay Description

Nano-Glo® Live Cell Reagent is a non-lytic detection reagent that enables dynamic real-time measurement of NanoLuc® or NanoBiT® activity. The detection reagent contains an optimized buffer system supplemented with the cell-permeable luciferase substrate furimazine. The assay reagent includes a proprietary component that reduces non-specific signals caused by autoluminescence, yielding a significantly better signal-to-background ratio.

Assay Features

Luciferase	NanoLuc® ; NanoBiT®
Assay type	Live assay; glow-type
Implementation	Homogeneous; 1-step
Substrate	Furimazine
Signal half-life	~ 2 h
Sample material	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well

References

Cannaert, A *et al.* (2016) Detection and Activity Profiling of Synthetic Cannabinoids and Their Metabolites with a Newly Developed Bioassay. Anal Chem. 88(23):11476-11485.

Oh-Hashi, K *et al.* (2016) SOD1 dimerization monitoring using a novel split-NanoLuc, NanoBit. Cell Biochem Funct. 34(7):497-504.

Dixon, AS *et al.* (2015) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. ACS Chemical Biology 19;11(2):400-8.

Product Box

Nano-Glo® Live Cell Assay System
Cat.# N2011, N2012, N2013

Real-time measurement of PKA subunit association and dissociation

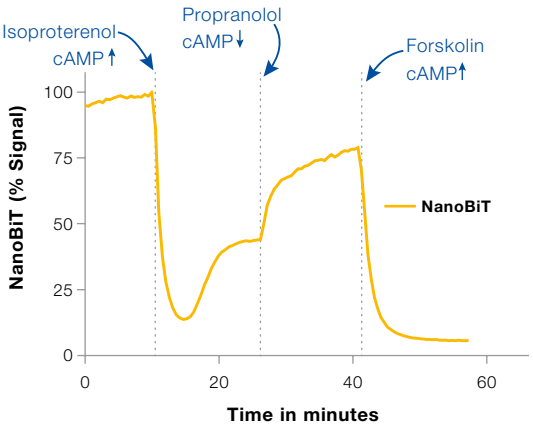


Fig. 7.1 Real-time measurement of protein kinase A (PKA) subunit association and dissociation using the Nano-Glo® Live Cell Assay System. HEK293 cells were transiently transfected with vectors encoding the regulatory and catalytic PKA subunits, respectively. The PKA subunits were fused to the NanoLuc® luciferase BiT fragments (SmBiT and LgBiT). The intracellular cAMP level was altered by adding positive (isoproterenol and forskolin) or negative (propanolol) modulators. The cAMP level negatively correlates with the association of the PKA subunits and thus complementation of the BiT fragments. Hence, an increase/decrease of the cAMP level results in an decrease/increase of the NanoBiT signal, respectively.

Microscopic imaging reveals the subcellular localization of the ß$_2$-adrenergic receptor (AR) fused to NanoLuc® luciferase

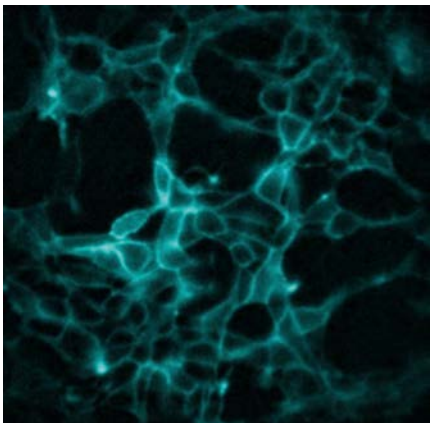


Fig. 7.2 Stable expression of a IL6-Nluc-ß$_2$AR fusion protein in HEK293 cells. Imaging of the cells was conducted after adding the Nano-Glo® Live Cell Assay System Reagent using an Olympus LV200 Bioluminescence Imager (exposure time: 5 sec).

NanoBRET™ Nano-Glo® Substrate

Application

Live-cell substrate for the detection of NanoLuc® activity in NanoBRET™ assays.

Assay Description

The NanoBRET™ Nano-Glo® Substrate is used to measure NanoLuc® luciferase donor signal in NanoBRET™ assays. The substrate yields a glow-type bioluminescent signal and is directly added to the cells. In standard NanoBRET™ assays the measurement is performed 2–3 min after addition of the NanoBRET™ Nano-Glo® Substrate. Single end-point measurements or kinetic measurements of up to 2 hours can be performed.

Assay Features

Luciferase	NanoLuc®
Assay type	Live assays, glow-type
Implementation	Homogeneous; 1-step
Substrate	Furimazine
Signal half-life	~ 2 h
Sample material	Mammalian cells
HTS capability	Tested in 96-, 384- and 1536-well

References

Machleidt, T *et al.* (2015) NanoBRET- A Novel BRET Platform for the Analysis of Protein:Protein Interactions. ACS Chem Biol. 10(8):1797-804.

Demont, EH *et al.* (2014) 1,3-Dimethyl Benzimidazolones Are Potent, Selective Inhibitors of the BRPF1 Bromodomain. ACS Med Chem Lett. 5(11):1190-5.

Robers, MB *et al.* (2015) Target engagement and drug residence time can be observed in living cells with BRET. Nat Commun. 3;6:10091.

Stoddart, LA *et al.* (2015) Application of BRET to monitor ligand binding to GPCRs. Nature Methods. 12(7):661-3.

Product Box

NanoBRET™ Nano-Glo® Substrate
Cat.# N1571, N1572, N1573

Application of NanoBRET™ Nano-Glo® Substrate in NanoBRET™ assays

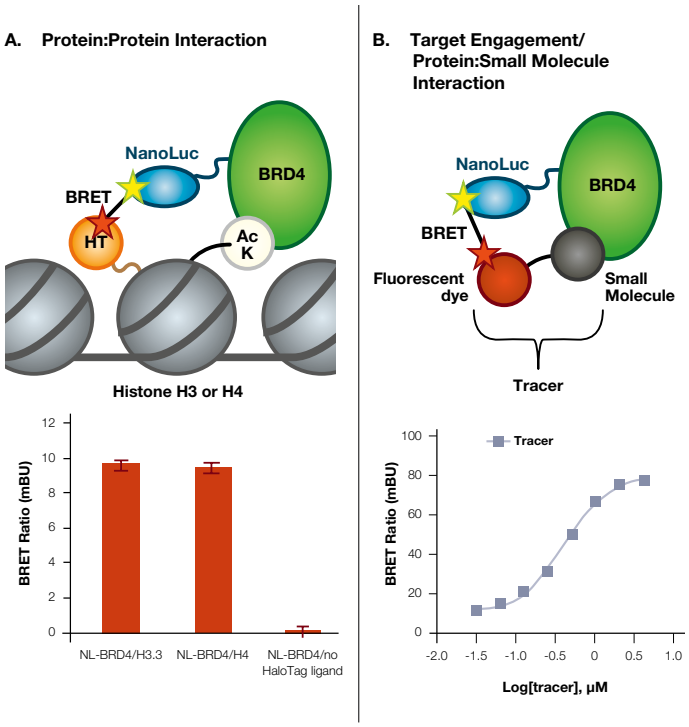


Fig. 7.3 The NanoBRET™ Nano-Glo® Substrate is used to dertermine NanoLuc® Luciferase donor signal in NanoBRET™ assays. Two experiments are shown: **(A)** Protein:protein interaction of BRD4 with histone H3- and H4-HaloTag® fusion proteins labeled with HaloTag® NanoBRET™ 618 Ligand. **(B)** Protein:small molecule interaction of the protein BRD4 and a small molecule coupled to the NanoBRET™ 618 Ligand acceptor fluorophore. BRD4 = Bromodomain-containing protein 4.

7.2 Live-Cell Substrates for the Detection of Firefly Luciferase

VivoGlo™ Luciferin, In Vivo Grade

Application

Detection of firefly luciferase activity in living systems.

Assay Description

VivoGlo™ Luciferin is a high-quality potassium salt of D-luciferin, ideally suited for the detection of firefly luciferase activity in living organisms.

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Implementation	Direct addition to medium or injection
Substrate	Potassium salt D-luciferin
Sample material	Living cells
Quality	Very low endotoxin levels ≤ 0.1EU/mg

References

Sawaengsak, C *et al.* (2014) Intranasal chitosan-DNA vaccines that protect across influenza virus subtypes. Int J Pharm. 473(1-2):113-25.

Keyaerts, M *et al.* (2012) Inhibition of firefly luciferase by general anesthetics: effect on in vitro and in vivo bioluminescence imaging. PLos ONE: 7(1):e30061.

Product Box

VivoGlo™ Luciferin, In Vivo Grade
Cat.# P1041, P1042, P1043

Luciferin-EF™ Endotoxin-Free Luciferin Na

Application

Detection of firefly luciferase activity in living systems for cell-based imaging applications.

Assay Description

Luciferin-EF™ Endotoxin-Free Luciferin Na contains a cell-permeable luciferin. The substrate does not exhibit autoluminescence and is stable in aqueous solutions. However, its limited half-life enables endpoint measurements only and no kinetics can be performed. Luciferin-EF™ is an endotoxin-free luciferin that is especially suited for living organisms.

Product Box

Luciferin-EF™ Endotoxin-Free Luciferin Na
Cat.# E6551, E6552

Assay Features

Luciferase	Firefly: luc, luc+, luc2
Implementation	Direct addition to medium or injection
Substrate	Sodium salt D-Luciferin
Signal half-life	20 min
Sample material	Living cells
Incubation time	2–20 min
Special features	Endotoxin-free

References

Jia, D *et al.* (2010) Development of a highly metastatic model that reveals a crucial role of fibronectin in lung cancer cell migration and invasion. BMC Cancer. 10:364.

Song, H *et al.* (2008) An immunotolerant HER-2/neu transgenic mouse model of metastatic breast cancer. Clin Cancer Res. 14(19):6116-24.

7.3 Live-Cell Substrates for the Detection of Renilla Luciferase

ViviRen™ Live Cell Substrate

Application

Detection of Renilla luciferase activity in living cells, kinetic measurements, BRET and imaging.

Assay Description

ViviRen™ Live Cell Substrate is used for the detection of Renilla luciferase activity in living cells. It contains a modified coelenterazine that is converted by intracellular esterases into coelenterazine-h. ViviRen™ Live Cell Substrate generates three- to fivefold brighter Renilla luciferase luminescence than wildtype coelenterazine. The signal-to-background ratio is up to 100-times higher than the wildtype coelenterazine. The ViviRen™ Substrate can be applied in a variety of Renilla-based assays for real-time measurements in multi-well plate formats.

Assay Features

Luciferase	Renilla: Rluc, hRluc
Substrate	Modified coelenterazine
Implementation	Addition to medium
Signal half-life	8–15 min
Sample material	Living cells
Incubation time	2 min

References

Kimura, T *et al.* (2010) Optimization of enzyme-substrate pairing for bioluminescence imaging of gene transfer using Renilla and Gaussia luciferases. J Gene Med.12(6):528-37.

Dimri, S *et al.* (2016) Use of BRET to Study Protein-Protein Interactions In Vitro and In Vivo. Methods Mol Biol. 1443:57-78.

Product Box

ViviRen™ Live Cell Substrate
Cat.# E6491, E6492, E6495

EnduRen™ Live Cell Substrate

Application

Detection of Renilla luciferase activity in living cells, kinetic measurements, BRET, cellular imaging.

Assay Description

EnduRen™ Substrate is applicable for kinetic measurements using Renilla luciferase. It contains a modified coelenterazine that is converted into coelenterazine-h by intracellular esterases. The substrate provides a very stable luminescence signal and allows measurements up to 24 hours after substrate addition.

Assay Features

Luciferase	Renilla: Rluc, hRluc
Implementation	Addition to medium
Substrate	Modified coelenterazine
Signal half-life	> 24 h
Sample material	Living cells
Incubation time	1.5 h

References

Farkas, T *et al.* (2016) Renilla Luciferase-LC3 Based Reporter Assay for Measuring Autophagic Flux. Methods in Enzymology.

Dragulescu-Andrasi, A *et al.* (2011) Bioluminescence resonance energy transfer (BRET) imaging of protein-protein interactions within deep tissues of living subjects. Proc. Natl. Acad. Sci. USA 108(29):12060-5.

Product Box

EnduRen™ Live Cell Substrate
Cat.# E6481, E6482, E6485

Scheme illustrating the uptake and the intracellular conversion of Renilla live-cell substrates

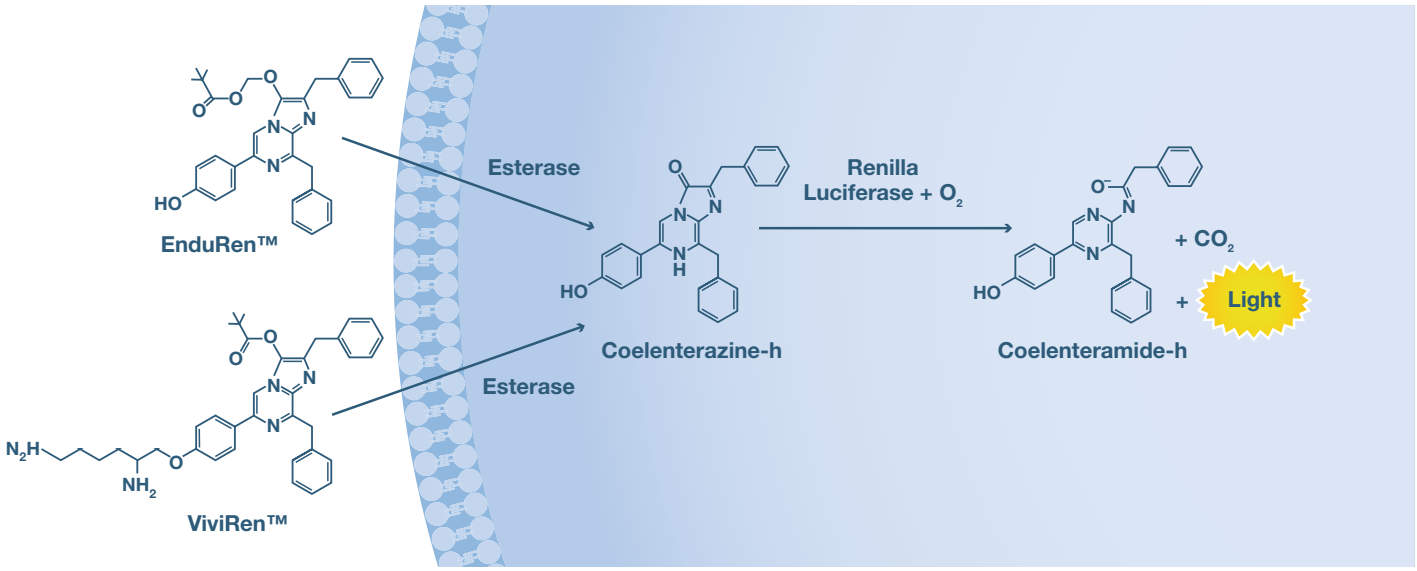


Fig. 7.4 ViviRen™ and EnduRen™ are converted to coelenterazine-h by intracellular esterases. Coelenterazine-h serves as substrate for the Renilla luciferase and is converted to coelenteramide-h. ViviRen™ allows measurements with a relatively short signal half-life and increased sensitivity. By contrast, EnduRen™ is stable for up to 24 hours and enables longterm measurements.

Tab. 7.1 Comparison of the live-cell substrates ViviRen™ and EnduRen™ for the measurement of Renilla luciferase activity

	ViviRen™	EnduRen™
Advantages	• Highest sensitivity • Extremely high signal-to-background ratio • Low autoluminecence in living cells • 3–5-times higher signal intensity compared to wild-type coelenterazine	• Kinetics measurements over 24 hours with a single substrate addition step • Minimal autoluminescence • Multiplexing with cell-based assays possible
Signal-to-background ratio	100-times higher compared to wild-type coelenterazine	10-times higher compared to wild-type coelenterazine
Signal half-life ($t_{1/2}$)	10–20 minutes	> 24 hours
Incubation time	2 minutes	1.5 hours
Application	• Short-term measurements	• Long-term kinetics

8 Detection with GloMax® Luminometers

Detection of Luminescence

The final important step in the luciferase reporter assay workflow is the detection of luminescence with an appropriate detection instrument. Undoubtedly, the right choice of an excellent detection instrument is key to successful reporter experiments. Instruments with poor **sensitivity** may miss low-level samples that could be the "hit" of interest. Instruments with a **narrow detection range** limit the accuracy or reproducibility of measurements. Finally, instruments that let the signal from bright wells spill into adjacent wells allow **crosstalk** to occur and skew experimental results. None of these issues are encountered when using GloMax® Luminometers. GloMax® Luminometers are ideally suited to record luminescence with high sensitivity over a broad detection range. This enables the measurements of weak and strong signals on the same plate. Well-to-well crosstalk is minimized thanks to a masking device integrated into the GloMax® Instruments.

For more information please visit
www.promega.de/products/fluorometers-luminometers-multimode-readers

GloMax® Discover und Explorer Systems

The **GloMax® Discover** System enables the detection of luminescence, fluorescence, UV/visible absorbance, BRET, FRET, and filtered luminescence with great sensitivity and a wide dynamic range using 6–384 well microplate formats. The instrument is operated by an integrated Tablet PC and can be either used as a standalone instrument or incorporated into an automated workflow. An electronic signature can be generated according to the FDA 21 CFR Part 11 regulation and an IQ/OQ qualification service can be performed on request. You prefer flexibility in terms of instrument configuration while keeping the possibility of an upgrade? As an alternative to the GloMax® Discover System, the novel GloMax® Explorer System offers modular design that can be easily adapted to your requirements. Two basic configurations are available with the opportunity to upgrade with visible absorbance.

GloMax® Navigator System

The GloMax® Navigator System is a 96-well plate luminometer that exhibits an extremely high sensitivity and minimal crosstalk. For the “bioluminescence lab” a broad application portfolio is offered, e.g. luciferase reporter gene measurements or luminescent cell viability assays. The system is easy to operate via the integrated Tablet PC. An electronic signature can be generated according to the FDA 21 CFR Part 11 regulation and an IQ/OQ qualification service can be performed on request.

GloMax® 20/20 Luminometer

The GloMax® 20/20 Luminometer can be applied for luminescence measurements in 1.5 mL reaction tubes or 35 mm dishes. Like all GloMax® instruments this luminometer is applicable to a broad range of luminescent reporter and cell viability assays.

Detection Instrument Selection Guide

www.promega.de/products/fluorometers-luminometers-multimode-readers/comparison/luminometers/

Comparison of GloMax® Systems

	GloMax® Discover System Cat.# GM3000 **GloMax® Explorer Systems** Cat.# GM3510, GM3500	**GloMax® Navigator System** Cat.# GM2000 (without injectors) GM2010 (with dual injectors)	**GloMax® 20/20 Luminometer** Cat.# E5311 (without injectors) E5321 (with single injector) E5331 (with dual injectors)
Detection modules	**GloMax® Discover (Cat.# GM3000):** luminescence, fluorescence, UV/Vis-Absorbance, filtered luminescence, BRET and FRET **GloMax® Explorer (Cat.# GM3510):** luminescence, fluorescence **GloMax® Explorer (Cat.# GM3500):** luminescence, fluorescence, Vis-absorbance	Luminescence	Luminescence, optional fluorescence
Sample format	6, 12, 24, 48, 96 and 384-well format (lid optional 96 and 384 well)	96-well format	1.5 ml reaction tubes 35 mm dish Minicell cuvette (fluorescence only)
Operation	Windows® Tablet PC with USB connection to instrument; Analysis software	Windows® Tablet-PC with USB connection to instrument; Analysis software	Integrated computer; Operation via touchscreen
Data	Saved in tablet software, data export via USB stick (Excel and CSV format), WLAN or local network	Saved in tablet software, data export via USB stick (Excel and CSV format), WLAN or local network	Saved in instrument, data export by serial interface to PC
Shaker	Functions linear or orbital, 100–500 cycles per minute	–	–
Heating system	Temperature range: room temperature to 45°C	–	–
Measurement speed	96-well plate: < 1 min 384-well plate: < 3 min	96-well plate: < 1 min	–
Automation capacity	Hardware and software accessible for external hardware/software control, including LIMS and SILA compatibility	–	–
Extras	Electronic signature according to FDA 21 CFR Part 11 regulation, IQ/OQ qualification service	Electronic signature according to FDA 21 CFR Part 11 regulation, IQ/OQ qualification service	–
Luminescence			
Detector	Photomultiplier for photon measurement (PMT), top-reading	Photomultiplier for photon measurement (PMT), top-reading	
Wavelength range	350–700 nm	350–700 nm	350–700 nm
Detection limit	3×10^{-21} mol luciferase	1.5×10^{-21} mol luciferase	$3x10^{-18}$ mol ATP or $1x10^{-21}$ mol luciferase
Linear range	9 decades	9 decades	> 8 decades
Crosstalk	Less than 3×10^{-5} (white 96-well plates, Corning Cat.# 3912)	Less than 3×10^{-5} (white 96-well plates, Corning Cat.# 3912)	–
Filtered luminecence	Integrated emission filters: 495 nm SP, 530 nm LP, 540 nm SP, 600 nm LP, 450 nm BP (8nm)	–	–

	GloMax® Discover System GloMax® Explorer Systems	GloMax® Navigator System	GloMax® 20/20 Luminometer
Fluorescence			
Detector	PIN photo diode, top-reading	–	Photomultiplier (PMT), bottom-reading
Light source	Wavelength-matched LED	–	Wavelength-matched LED
Standard filter	UV (Ex: 365 nm, Em: 415–445 nm); Blue (Ex: 475 nm, Em: 500–550 nm); Green (Ex: 525 nm, Em: 580–640 nm); Red (Ex: 625 nm, Em: 660–720 nm) AFC (Ex: 405 nm, Em: 495–505 nm); customer specific filters on request	–	Fluorescence modules optionally available UV (Ex: 365 nm, Em: 440–470 nm); Blue (Ex: 460 nm, Em: 515–575 nm)
Detection limit	2 fmol fluorescein/200 µl	–	10 ng/ml dsDNA (Hoechst Dye 33258) 450 pg/ml dsDNA (DNA quantification dye)
Linear range	> 6 decades (assay dependent)	–	5 decades (assay dependent)
UV and Vis Absorbance			
Detector	Photomultiplier (PMT), top-reading,	–	–
Light source	Xenon lamp	–	–
Spectrum	200–600 nm	–	–
Filters	**GloMax® Discover:** 230, 260, 280, 320, 405, 450, 490, 560 and 600 nm with 10 nm bandwidth each **GloMax® Explorer:** 405, 450, 490, 560 und 600 nm with 10 nm bandwidth each	–	–
Detection limit	0.1 OD	–	–
Linear range	0–4 OD	–	–
Injector System			
Injector system	Dual injectors (optional)	Dual injectors (optional)	Single- or Dual-injectors (optional)
Dispension volume	5–200 µl in 1µl steps	5–200 µl in 1 µl steps	25-300 µl in 5 µl steps
Plate formats	6, 12, 24, 48, and 96-well plates	96-well plates	Single tube
Injection speed	20–500 µl per second	20–500 µl per second	200 µl per second
Void volume	500 µl	500 µl	750 µl

9 Product Overview

9.1 Reporter Vectors

Vector	Reporter gene	Promoter	PEST sequence	Selection marker	Cat. No.
Firefly reporter vectors					
Promoter vectors*					
pGL4.10[luc2]	luc2	-	-	-	E6651
pGL4.11[luc2P]	luc2P	-	hPEST	-	E6661
pGL4.14[luc2/Hygro]	luc2	-	-	Hygro	E6691
pGL4.15[luc2P/Hygro]	luc2P	-	hPEST	Hygro	E6701
pGL4.17[luc2/Neo]	luc2	-	-	Neo	E6721
pGL4.18[luc2P/Neo]	luc2P	-	hPEST	Neo	E6731
pGL4.20[luc2/Puro]	luc2	-	-	Puro	E6751
pGL4.21[luc2P/Puro]	luc2P	-	hPEST	Puro	E6761
Vectors with minimal promoter					
pGL4.23[luc2/minP]	luc2	minP	-	-	E8411
pGL4.24[luc2P/minP]	luc2P	minP	hPEST	-	E8421
pGL4.26[luc2/minP/Hygro]	luc2	minP	-	Hygro	E8441
pGL4.27[luc2P/minP/Hygro]	luc2P	minP	hPEST	Hygro	E8451
Fusion vectors					
pSP-luc+NF Fusion Vector (N-Term)	luc+	SP6 (sense); T7 (antisense)	-	-	E4471
Control vectors					
pGL4.13[luc2/SV40]	luc2	SV40	-	-	E6681
pGL4.50[luc2/CMV/Hygro]	luc2	CMV	-	Hygro	E1310
pGL4.51[luc2/CMV/Neo]	luc2	CMV	-	Neo	E1320
pGL4.53[luc2/PGK]	luc2	PGK	-	-	E5011
pGL4.54[luc2/TK]	luc2	TK	-	-	E5061
Renilla reporter vectors					
Promoterless* vectors					
pGL4.70[hRluc]	hRluc	-	-	-	E6881
pGL4.71[hRlucP]	hRlucP	-	hPEST	-	E6891
pGL4.76[hRluc/Hygro]	hRluc	-	-	Hygro	E6941
pGL4.77[hRlucP/Hygro]	hRlucP	-	hPEST	Hygro	E6951
pGL4.79[hRluc/Neo]	hRluc	-	-	Neo	E6971
pGL4.80[hRlucP/Neo]	hRlucP	-	hPEST	Neo	E6981
pGL4.82[hRluc/Puro]	hRluc	-	-	Puro	E7501
pGL4.83[hRlucP/Puro]	hRlucP	-	hPEST	Puro	E7511
pRL-null	Rluc**	-	-	-	E2271
Vectors with minimal promoter					
pGL4[hRlucP/minP/Hygro]	hRlucP	minP	hPEST	Hygro	CS182201
Control vectors					
pGL4.73[hRluc/SV40]	hRluc	SV40	-	-	E6911
pGL4.74[hRluc/TK]	hRluc	TK	-	-	E6921
pGL4.75[hRluc/CMV]	hRluc	CMV	-	-	E6931
pRL-CMV	Rluc**	CMV	-	-	E2261
pRL-SV40	Rluc**	SV40	-	-	E2231
pRL-TK	Rluc**	TK	-	-	E2241

** wild-type Renilla luciferase

9.1 Reporter Vectors (continued)

Vector	Reporter gene	Promoter	PEST sequence	Selection marker	Cat. No.
NanoLuc® reporter vectors					
Promoterless vectors					
pNL1.1[*Nluc*]	Nluc	-	-	-	N1001
pNL1.2[*NlucP*]	NlucP	-	hPEST	-	N1011
pNL1.3[*secNluc*]	secNluc	-	-	-	N1021
pNL2.1[*Nluc/Hygro*]	Nluc	-	-	Hygro	N1061
pNL2.2[*NlucP/Hygro*]	NlucP	-	hPEST	Hygro	N1071
pNL2.3[*secNluc/Hygro*]	secNluc	-	-	Hygro	N1081
Vectors with minimal promoter					
pNL3.1[*Nluc*/minP]	Nluc	minP	-	-	N1031
pNL3.2[*NlucP*/minP]	NlucP	minP	hPEST	-	N1041
pNL3.3[*secNluc*/minP]	secNluc	minP	-	-	N1051
pNL[NlucP/minP/Hygro]	NlucP	minP	hPEST	Hygro	CS188006
Control vectors					
pNL1.1.CMV[*Nluc*/CMV]	Nluc	CMV	-	-	N1091
pNL1.1.PGK[Nluc/PGK] Vector	Nluc	PKG	-	-	N1441
pNL1.1.TK[Nluc/TK]Vector	Nluc	TK	-	-	N1501
pNL1.3.CMV [*secNluc*/CMV]	secNluc	CMV	-	-	N1101
pNL3.2.CMV[NlucP/CMV]	NlucP	CMV	hPEST	-	N1411
Cloning vectors for the generation of custom NanoLuc® fusion vectors					
Fusion vectors with MCS sites					
pNLF1-C [CMV/Hygro] (C-Term)	Nluc	CMV	-	Hygro	N1361
pNLF1-N [CMV/Hygro] (N-Term)	Nluc	CMV	-	Hygro	N1351
pNLF1-secN [CMV/Hygro] (N-Term)	secNluc	CMV	-	Hygro	N1371
Fusion vectors with Flexi® sites					
pFN31A Nluc CMV-Hygro Flexi® Vector (N-Term)	Nluc	CMV	-	Hygro	N1311
pFN31K Nluc CMV-neo Flexi® Vector (N-Term)	Nluc	CMV	-	Neo	N1321
pFC32A Nluc CMV-Hygro Flexi® Vector (C-Term)	Nluc	CMV	-	Hygro	N1331
pFC32K Nluc CMV-neo Flexi® Vector (C-Term)	Nluc	CMV	-	Neo	N1341
NanoLuc® stability sensors					
p[p53-Nluc/CMV/Neo]	Nluc-p53	CMV	-	Neo	CS179001
pNLF1-NRF2 [CMV/neo]	Nluc/NRF2	CMV	-	Neo	N1391
pNLF1-HIF1A [CMV/neo]	Nluc/HiF1A	CMV	-	Neo	N1381
refer to **NanoLuc® fusion vectors** for cloning of custom stability sensors					
Coincidence reporters					
pNLCol1[luc2-P2A-NlucP/Hygro]	luc2, NlucP		hPEST	Hygro	N1461
pNLCol2[luc2-P2A-NlucP/minP/Hygro]	luc2, NlucP		hPEST	Hygro	N1471
pNLCol3[luc2-P2A-NlucP/CMV/Hygro]	luc2, NlucP		hPEST	Hygro	N1481
pNLCol4[luc2-P2A-NlucP/PGK/Hygro]	luc2, NlucP		hPEST	Hygro	N1491
RNA interference					
pmirGLO Vector	luc2, hRluc	PGK, SV40	-	Neo	E1330
pmirNanoGlo Vector	luc2, NlucP	SV40, PGK	PEST	Blasticidin	CS194105
psiCHECK™-2 Vector	hRluc, hluc+	SV40, HSV-TK	-	-	C8021

9.1 Reporter Vectors (continued)

Activator/Pathway	Transcription Factor	Response Element/ Promoter	Nluc Vector	Nluc Cat.#	Fluc Vector	Firefly Cat.#
Pathway vectors for GPCR signaling and cellular stress response						
Oxidativer Stress	Nrf2	ARE	pNL[NlucP/ARE/Hygro]	CS180902	pGL4.37[luc2P/ARE/Hygro]	E3641
cAMP/PKA	CREB	CRE	pNL[NlucP/CRE/Hygro]	CS186804	pGL4.29[luc2P/CRE/Hygro]	E8471
Calcium/Calcineurin	NFAT	NFAT-RE	pNL[NlucP/NFAT-RE/Hygro]	CS177602	pGL4.30[luc2P/NFAT-RE/Hygro]	E8481
NF-κB	NFκB	NFκB-RE	pNL3.2[NlucP/NFκB-RE/Hygro]	N1111	pGL4.32[luc2P/NFκB-RE/Hygro]	E8491
RhoA (Gα12/13)	SRF	SRF-RE	pNL[NlucP/SRF/Hygro]	CS194101	pGL4.34[luc2P/SRF-RE/Hygro]	E1350
DNA Damage/p53	p53	p53-RE	pNL[NlucP/p53-RE/Hygro]	CS194102	pGL4.38[luc2P/p53 RE/Hygro]	E3651
Endoplasmic Reticulum Stress	ATF4	ATF4 ERSE	pNL[NlucP/ATF4-RE/Hygro]	CS183701	pGL4[luc2P/ATF4-RE/Hygro]	CS180501
Endoplasmic Reticulum Stress	ATF6	ATF6 ERSE	pNL[NlucP/ATF6-RE/Hygro]	CS186805	pGL4.39[luc2P/ATF6 RE/Hygro]	E3661
Heat shock	HSF1	HSE	-	-	pGL4.41[luc2P/HSE/Hygro]	E3751
Hypoxia	HiF1α	HRE	pNL[NlucP/HRE/Hygro]	CS180901	pGL4.42[luc2P/HRE/Hygro]	E4001
Xenobiotic Stress	AhR	XRE	pNL[NlucP/XRE/Hygro]	CSxxxxxx	pGL4.43[luc2P/XRE/Hygro]	E4121
MAPK/JNK	AP1	AP1 RE	pNL[NlucP/AP1-RE/Hygro]	CS177603	pGL4.44[luc2P/AP1 RE/Hygro]	E4111
Myc, PI3K/Akt/MAPK	Myc/Max	Myc RE	pNL[NlucP/MycMax-RE/Hygro]	CS175201	pGL4[luc2P/Myc/Hygro]	CS180201
Androgen Activation in Prostate Cancer	-	Human PSA Promoter	-	-	pGL4[luc2P/PSA-long/Hygro]	CS181504
Cytochrome P450/ drug metabolism	-	Human 3A4	-	-	pGL4[luc2P/3A4/Hygro]	CS185401
Cytochrome P450/ drug metabolism	-	Human 2B6	-	-	pGL4[luc2P/2B6/Hygro]	CS185402
Ras/MEK-1	SRE	SRE	pNL[NlucP/SRE/Hygro]	CS177601	pGL4.33[luc2P/SRE/Hygro]	E1340
Pathway vectors for cytokine signaling						
JAK/STAT1/2 IFN-α	STAT1:STAT2	ISRE	pNL[NlucP/ISRE/Hygro]	CS190901	pGL4.45[luc2P/ISRE/Hygro]	E4141
JAK/STAT3 IL-6	STAT3:STAT3	SIE	pNL[NlucP/SIE/Hygro]	CS189701	pGL4.47[luc2P/SIE/Hygro]	E4041
TGF-β	SMAD3:SMAD4	SBE	pNL[NlucP/SBE/Hygro]	CS177101	pGL4.48[luc2P/SBE/Hygro]	E3671
Wnt	TCF/LEF	TCF-LEF RE	pNL[NlucP/TCF/LEF-RE/Hygro]	CS181801	pGL4.49[luc2P/TCF-LEF-RE/Hygro]	E4611
JAK/STAT5 IL3	STAT5:STAT5	STAT5-RE	pNL[NlucP/STAT5-RE/Hygro]	CS180903	pGL4.52[luc2P/STAT5-RE/Hygro]	E4651
Hedgehog	Gli	Gli-RE	-	-	pGL4[luc2P/Gli-RE/Hygro]	CS171301
JAK/STAT1 IFN-γ	IFN-γ activation site	GAS RE	pNL[NlucP/GAS-RE/Hygro]	CS188003	pGL4[luc2P/C/EBP-RE/Hygro]	CS185201
Multiple	C/EBP	C/EBP-RE	pNL[NlucP/CEBP-RE/Hygro]	CS188003	pGL4[luc2P/C/EBP-RE/Hygro]	CS185201
JAK/STAT4 IL12	Response Element	IRF1-RE	-	-	pGL4[luc2P/STAT4-RE/Hygro]	CS181501

9.2 Transfection Reagents

Product	Size	Cat. No.
Transfection reagents		
TransFast™ Transfection Reagent	1.2 mg	E2431
FuGENE® HD Transfection Reagent	1 ml	E2311
FuGENE® HD Transfection Reagent	5 x 1 ml	E2312
FuGENE® 6 Transfection Reagent	1 ml	E2691
FuGENE® 6 Transfection Reagent	5 x 1 ml	E2692
FuGENE® 6 Transfection Reagent	0.5 ml	E2693
ViaFect™ Transfection Reagent	0.75 ml	E4981
ViaFect™ Transfection Reagent	2 x 0.75 ml	E4982

9.3 Luciferase Assays

Product	Size	Cat. No.
Single reporter gene assays (lytic; endpoint)		
NanoLuc® assays		
Nano-Glo® Luciferase Assay	10 ml	N1110
Nano-Glo® Luciferase Assay	100 ml	N1120
Nano-Glo® Luciferase Assay	10 x 10 ml	N1130
Nano-Glo® Luciferase Assay	10 x 100 ml	N1150
Firefly assays		
Luciferase Assay Reagent	100 ml	E1483
Luciferase Assay System	100 assays	E1500
Luciferase Assay System, 10-Pack	1000 assays	E1501
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay System Freezer Pack	1000 assays	E4530
Luciferase 1000 Assay System	1000 assays	E4550
Luciferase Cell Culture Lysis 5X Reagent	30 ml	E1531
Reporter Lysis 5X Buffer	30 ml	E3971
Bright-Glo™ Luciferase Assay System	10 ml	E2610
Bright-Glo™ Luciferase Assay System	100 ml	E2620
Bright-Glo™ Luciferase Assay System	10 x 100 ml	E2650
ONE Glo™ Luciferase Assay System	10 ml	E6110
ONE Glo™ Luciferase Assay System	100 ml	E6120
ONE Glo™ Luciferase Assay System	1 L	E6130
ONE-Glo™ EX Luciferase Assay System	10 ml	E8110
ONE-Glo™ EX Luciferase Assay System	100 ml	E8120
ONE-Glo™ EX Luciferase Assay System	10 x 10 ml	E8130
ONE-Glo™ EX Luciferase Assay System	10 x 100 ml	E8150
Steady-Glo® Luciferase Assay System	10 ml	E2510
Steady-Glo® Luciferase Assay System	100 ml	E2520
Steady-Glo® Luciferase Assay System	10 x 100 ml	E2550
Renilla assays		
Renilla Luciferase Assay System	100 assays	E2810
Renilla Luciferase Assay System	1000 assays	E2820
Renilla-Glo® Luciferase Assay System	10 ml	E2710
Renilla-Glo® Luciferase Assay System	100 ml	E2720
Renilla-Glo® Luciferase Assay System	10 x 100 ml	E2750

9.3 Luciferase Assays (continued)

Product	Size	Cat. No.
Dual reporter gene assays (lytic; endpoint)		
Dual assays for NanoLuc® + Firefly Luciferase		
Nano-Glo® Dual-Luciferase® Reporter Assay System	10 ml	N1610
Nano-Glo® Dual-Luciferase® Reporter Assay System	100 ml	N1620
Nano-Glo® Dual-Luciferase® Reporter Assay System	10 x 10 ml	N1630
Nano-Glo® Dual-Luciferase® Reporter Assay System	10 x 100 ml	N1650
Nano-Glo® Dual-Luciferase® Reporter Assay/pNL1.1.TK Bundle	1x	N1521
Nano-Glo® Dual-Luciferase® Reporter Assay/pNL1.1.PGK Bundle	1x	N1531
Nano-Glo® Dual-Luciferase® Reporter Assay/pGL4.54[luc2/TK] Bundle	1x	N1541
Nano-Glo® Dual-Luciferase® Reporter Assay/pGL4.53[luc2/PGK] Bundle	1x	N1551
NanoDLR™/pNL1.1.TK Helix® Bundle	1x	N1561
Passive Lysis 5X Buffer	30 ml	E1941
Dual Assays for Firefly + Renilla Luciferase		
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-Pack	1000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System	1000 assays	E1980
Passive Lysis 5X Buffer	30 ml	E1941
Dual-Glo® Luciferase Assay System	10 ml	E2920
Dual-Glo® Luciferase Assay System	100 ml	E2940
Dual-Glo® Luciferase Assay System	10 x 100 ml	E2980
Chroma-Luc™ Luciferase		
Chroma-Glo™ Luciferase Assay System	10 ml	E4910
Chroma-Glo™ Luciferase Assay System	100 ml	E4920
Multiplex Assays (cell viability & firefly activity)		
ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay	1 plate	E7110
ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay	10 plates	E7120

Product	Size	Cat. No.
Live-cell subtrates for dynamic real-time measurements		
NanoLuc® Live Cell Assays		
Nano-Glo® Live Cell Assay System	100 assays	N2011
Nano-Glo® Live Cell Assay System	1000 assays	N2012
Nano-Glo® Live Cell Assay System	10000 assays	N2013
NanoBRET™ Nano-Glo® Substrate	50 µl	N1571
NanoBRET™ Nano-Glo® Substrate	5 x 50 µl	N1572
NanoBRET™ Nano-Glo® Substrate	2 x 1.25 ml	N1573
Firefly Live Cell Assays		
Luciferin-EF™	25 mg	E6551
Luciferin-EF™	250 mg	E6552
VivoGlo™ Luciferin, In Vivo Grade	50 mg	P1041
VivoGlo™ Luciferin, In Vivo Grade	250 mg	P1042
VivoGlo™ Luciferin, In Vivo Grade	1 g	P1043
Renilla Live Cell Assays		
EnduRen™ Live Cell Substrate	0.34 mg	E6481
EnduRen™ Live Cell Substrate	3.4 mg	E6482
EnduRen™ Live Cell Substrate	34 mg	E6485
ViviRen™ Live Cell Substrate	0.37 mg	E6491
ViviRen™ Live Cell Substrate	3.7 mg	E6492
ViviRen™ Live Cell Substrate	37 mg	E6495
EnduRen™ In Vivo Renilla Luciferase Substrate	0.34 mg	P1111
EnduRen™ In Vivo Renilla Luciferase Substrate	3.4 mg	P1112
ViviRen™ In Vivo Renilla Luciferase Substrate	0.37 mg	P1231
ViviRen™ In Vivo Renilla Luciferase Substrate	3.7 mg	P1232
Other substrates		
VivoGlo™ Caspase-3/7 Substrate (Z-DEVD-Aminoluciferin)	50 mg	P1781
VivoGlo™ Caspase-3/7 Substrate (Z-DEVD-Aminoluciferin)	5 x 50 mg	P1782
VivoGlo™ Luciferin-β-Galactoside Substrate (6-O-β-galactopyranosyl luciferin)	50 mg	P1061
VivoGlo™ Luciferin-β-Galactoside Substrate (6-O-β-galactopyranosyl luciferin)	250 mg	P1062
Beetle Luciferin, Potassium Salt	5 mg	E1601
Beetle Luciferin, Potassium Salt	50 mg	E1602
Beetle Luciferin, Potassium Salt	250 mg	E1603
Beetle Luciferin, Potassium Salt	1 g	E1605
Coelenterazine	250 µg	S2001
Coelenterazine-h	250 µg	S2011

9.4 NanoBRET™ PPI Assays

Product	Size	Cat. No.
"Ready-to-use" NanoBRET™ assays		
NanoBRET™ BRD4/ Histone H3.3 Interaction Assay	1x	N1830
NanoBRET™ BRD4/ Histone H4 Interaction Assay	1x	N1890
NanoBRET™ BRD9/ Histone H3.3 Interaction Assay	1x	N1840
NanoBRET™ BRD9/Histone H4 Interaction Assay	1x	N1900
NanoBRET™ BRPF1/ Histone H3.3 Interaction Assay	1x	N1860
NanoBRET™ BRPF1/ Histone H4 Interaction Assay	1x	N1910
NanoBRET™ cMyc/MAX Interaction Assay	1x	N1870
NanoBRET™ Kras/Braf Interaction Assay	1x	N1880
NanoBRET™ PPI Control Pair (p53, MDM2)	2 x 20 µg	N1641

for additional **"Ready-to-use" NanoBRET™ assays** refer to www.promega.com/nanobret
Beside the optimized vectors constructs, all "Ready-to-use" NanoBRET™ assays contain the p53/MDM2 control vector pair and enough reagent to perform 200 96-well assays

NanoBRET™ Starter Kits		
NanoBRET™ PPI Flexi® Starter System	**1x**	**N1821**
N1321: pFN31K Nluc CMV-neo Flexi® Vector		
N1341: pFC32K Nluc CMV-neo Flexi® Vector		
G2821: pFN21A HaloTag® CMV Flexi® Vector		
G9661: pFC14K HaloTag® CMV Flexi® Vector		
N1641: NanoBRET™ PPI Control Pair (p53, MDM2)		
N1661: NanoBRET™ Nano-Glo® Detection System (200 assays, 96-well)		
NanoBRET™ PPI MCS Starter System	**1x**	**N1811**
N1351: pNLF1-N [CMV/Hygro] Vector		
N1361: pNLF1-C [CMV/Hygro] Vector		
G7721: pHTN HaloTag® CMV-neo Vector		
G7711: pHTC HaloTag® CMV-neo Vector		
N1641: NanoBRET™ PPI Control Pair (p53, MDM2)		
N1661: NanoBRET™ Nano-Glo® Detection System (200 assays, 96-well)		

Fusion vectors for extracellular localization	
pFN210A SSHaloTag® CMVneoFlexi® Vector	CS199702
pFCsecNluc_CMV_Neo_Flexi® Vector	CSxxxxx
pNLF1-secN [CMV/Hygro] (N-Term)	N1371

Product	Size	Cat. No.
NanoBRET™ Nano-Glo® Detection Reagents		
NanoBRET™ Nano-Glo® Detection System	200 assays	N1661
NanoBRET™ Nano-Glo® Detection System	1000 assays	N1662
NanoBRET™ Nano-Glo® Detection System	10000 assays	N1663
NanoBRET™ Nano-Glo® Substrate		
NanoBRET™ Nano-Glo® Substrate	50 µl	N1571
NanoBRET™ Nano-Glo® Substrate	5 x 50 µl	N1572
NanoBRET™ Nano-Glo® Substrate	2 x 1.25 ml	N1573
Separately available		
NanoBRET™ Positive Control (Nluc-HaloTag Fusion)	2 x 20 µg	N1581
NanoBRET™ PPI Control Pair (p53, MDM2)	2 x 20 µg	N1641
HaloTag® NanoBRET™ 618 Ligand	20 µl	G9801

9.5 NanoBRET™ TE Assays

Product	Size	Cat. No.
NanoBRET ™ Target Engagement for HDACs		
NanoBRET™ TE Intracellular HDAC Assay	100 assays	N2080
NanoBRET™ TE Intracellular HDAC Assay	1000 assays	N2081
NanoBRET™ TE Intracellular HDAC Detection Reagents	10000 assays	N2090
NanoBRET™ TE HDAC DNA Bundle	1 x	N2120
NanoBRET™ TE Intracellular HDAC Complete Kit	1000 assays	N2170
NanoBRET™ Target Engagement Assays for BET/BRD		
NanoBRET™ TE Intracellular BET BRD Assay	100 assays	N2130
NanoBRET™ TE Intracellular BET BRD Assay	1000 assays	N2131
NanoBRET™ TE Intracellular BET BRD Detection Reagents	10000 assays	N2140
NanoBRET™ TE BET BRD DNA Bundle	1 x	N2150
NanoBRET™ TE Intracellular BET BRD Complete Kit	1000 assays	N2180
NanoBRET™ Target Engagement Assays for Kinases*		
NanoBRET™ TE Intracellular Kinase Assay, K-4	100 assays	N2520
NanoBRET™ TE Intracellular Kinase Assay, K-4	1000 assays	N2521
NanoBRET™ TE Intracellular Kinase Assay, K-4	10000 assays	N2540
NanoBRET™ TE Intracellular Kinase Assay, K-5	100 assays	N2500
NanoBRET™ TE Intracellular Kinase Assay, K-5	1000 assays	N2501
NanoBRET™ TE Intracellular Kinase Assay, K-5	10000 assays	N2530
Separately available		
Intracellular TE Nano-Glo® Substrate/Inhibitor	1000 assays	N2160
Intracellular TE Nano-Glo® Substrate/Inhibitor	10000 assays	N2161
Tracer Dilution Buffer	50 ml	N2191
Transfection Carrier DNA	5 x 20 µg	E4881
Fluorescent dyes for the generation of tracers		
NanoBRET™ 618 TFP	5 mg	CS189403
NanoBRET™ 590 SE	5 mg	CS189404

* Please visit our website for a list of 127 “ready-to-use” human kinase vector constructs used either with NanoBRET™ TE K-4 or NanoBRET™ TE K-5

9.6 NanoBiT™ PPI Assays

Product	Size	Cat. No.
NanoBiT™ Starter Kits		
NanoBiT™ PPI MCS Starter System	1x	N2014
2 x 125 µl Nano-Glo® Live Cell Substrate		
2 x 2.5 ml Nano-Glo® LCS Dilution Buffer		
20 µg pBiT1.1-C [TK/LgBiT] Vector		
20 µg pBiT2.1-C [TK/SmBiT] Vector		
20 µg pBiT1.1-N [TK/LgBiT] Vector		
20 µg pBiT2.1-N [TK/SmBiT] Vector		
20 µg SmBiT-PRKACA Control Vector		
20 µg LgBiT-PRKA2A Control Vector		
20 µg NanoBiT™ Negative Control Vector (HaloTag-SmBiT)		
NanoBiT™ PPI Flexi® Starter System	1x	N2015
2 x 125 µl Nano-Glo® Live Cell Substrate		
2 x 2.5 ml Nano-Glo® LCS Dilution Buffer		
20 µg pFC34K LgBiT TK-neo Flexi® Vector		
20 µg pFC36K SmBiT TK-neo Flexi® Vector		
20 µg pFN33K LgBiT TK-neo Flexi® Vector		
20 µg pFN35K SmBiT TK-neo Flexi® Vector		
20 µg SmBiT-PRKACA Control Vector		
20 µg LgBiT-PRKA2A Control Vector		
20 µg NanoBiT™ Negative Control Vector (HaloTag-SmBiT)		
20 µg pF4A CMV Flexi® Vector (= Entry Vector)		
NanoBiT™ control pairs		
NanoBiT™ PPI Control Pair (FKBP, FRB)	1x	N2016
NanoBiT™ PPI Control Pair (MDM2, p53)	1x	CS1603B09
NanoBiT™ Nano-Glo® Detection Reagent		
Nano-Glo® Live Cell Assay System	100 assays	N2011
Nano-Glo® Live Cell Assay System	1000 assays	N2012
Nano-Glo® Live Cell Assay System	10000 assays	N2013
Additional NanoBiT™ cloning vectors		
NanoBiT™ PPI CMV MCS vector pack (4x vectors)	1x	CS1603B23
pBiT1.2-C [CMV/LgBiT] Vector	1x	CS1603B23A
pBiT2.2-C [CMV/SmBiT] Vector	1x	CS1603B23B
pBiT1.2-N [CMV/LgBiT] Vector	1x	CS1603B23C
pBiT2.2-N [CMV/SmBiT] Vector	1x	CS1603B23D
NanoBiT™ PPI CMV Flexi vector pack (4x vectors)	1x	CS1603B24
pFN213K LgBiT CMV-neo Flexi Vector	1x	CS1603B24A
pFN214K SmBiT CMV-neo Flexi Vector	1x	CS1603B24B
pFC215K LgBiT CMV-neo Flexi Vector	1x	CS1603B24C
pFC216K SmBiT CMV-neo Flexi Vector	1x	CS1603B24D

9.7 HiBiT Vectors and Detection

Product	Size	Cat. No.
HiBiT Cloning Vectors		
pBiT3.1-N [CMV/HiBiT/Blast] Vector	1 x 20µg	N2361
pBiT3.1-C [CMV/HiBiT/Blast] Vector	1 x 20µg	N2371
pBiT3.1-secN [CMV/HiBiT/Blast] Vector	1 x 20µg	N2381
pFC37K HiBiT CMV-neo Flexi® Vector	1 x 20µg	N2391
pFN38K HiBiT CMV-neo Flexi® Vector	1 x 20µg	N2401
pFN39K secHiBiT CMV-neo Flexi® Vector	1 x 20µg	N2411
HiBiT Detection Reagents		
Nano-Glo® HiBiT Blotting System	100ml	N2410
Nano-Glo® HiBiT Extracellular Detection System	10ml	N2420
Nano-Glo® HiBiT Extracellular Detection System	100ml	N2421
Nano-Glo® HiBiT Extracellular Detection System	10 x 100ml	N2422
Nano-Glo® HiBiT Lytic Detection System	10ml	N3030
Nano-Glo® HiBiT Lytic Detection System	100ml	N3040
Nano-Glo® HiBiT Lytic Detection System	10 x 100ml	N3050

9.8 GloSensor™ Assays

Product	Size	Cat. No.
Detection of cAMP		
GloSensor™ cAMP HEK293 Cell Line	2 vials	E1261
pGloSensor™-22F cAMP Plasmid	20 µg	E2301
pGloSensor™-20F cAMP Plasmid	20 µg	E1171
GloSensor™ cAMP Reagent	25 mg	E1290
Detection of cGMP		
GloSensor-40F cGMP HEK293 Cell line	2 vials	CS182801
GloSensor-42F cGMP HEK293 Cell line	2 vials	CS182802
pGloSensor-40F cGMP Plasmid	20 µg	CS178901
pGloSensor-42F cGMP Plasmid	20 µg	CS177001
GloSensor™ cAMP Reagent	25 mg	E1290
Detection of protease activity		
pGloSensor-30 DEVDG Caspase Plasmid	20 µg	CS182101
GloSensor™ cAMP Reagent	25 mg	E1290
Protease-Glo™ Assay	Starter kit	G9451
pGloSensor™-10F Linear Vector	1 µg	G9461
Bright-Glo™ Luciferase Assay System	10 ml	E2650

9.9 GloMax® Detection Instruments

Product	Cat. No.
GloMax® Discover System	GM3000
GloMax® Explorer Fully Loaded Model	GM3500
GloMax® Explorer with Luminescence and Fluorescence	GM3510
GloMax® Navigator System	GM2000
GloMax® Navigator System with Dual Injectors	GM2010
GloMax® 20/20 Luminometer	E5311
GloMax® 20/20 Luminometer w/Single Auto-Injector	E5321
GloMax® 20/20 Luminometer w/Dual Auto-Injector	E5331

Please visit our website for GloMax® Accessories products: www.promega.com

Product Use Limitations, Warranty, Disclaimer

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact. Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications. Product claims are subject to change. Please contact Promega Technical Services (techserv@promega.com) or access the Promega online catalog (www.promega.com/catalog/) for the most up-to-date information on Promega products.

Contact Promega

North America

United States/Canada
Tel: 608-274-4330
Fax: 608-277-2516
Toll-Free Tel: 800 356 9526
Toll-Free Fax: 800 356 1970
E-mail: custserv@promega.com

South America

Brazil
Tel: +55 11 5090 3780
Fax: +55 11 5096 3780
E-mail: promega.brasil@promega.com

Europe

Germany/Austria
Tel: +49 6227 6906 0
Fax: +49 6227 6906 222
E-mail: de_custserv@promega.com

Switzerland
Tel: +44 878 90 00
Fax: +44 878 90 10
E-mail: ch_custserv@promega.com

France
Tel: +33 0437 2250 00
Fax: +33 0437 2250 10
Numero Vert: 0 800 48 79 99
E-mail: contactfr@promega.com

Italy
Tel: +39 0254 0501 94
Fax: +39 0256 5616 45
Toll-Free Phone: 800 6918 18
E-mail: customerservice.italia@promega.com

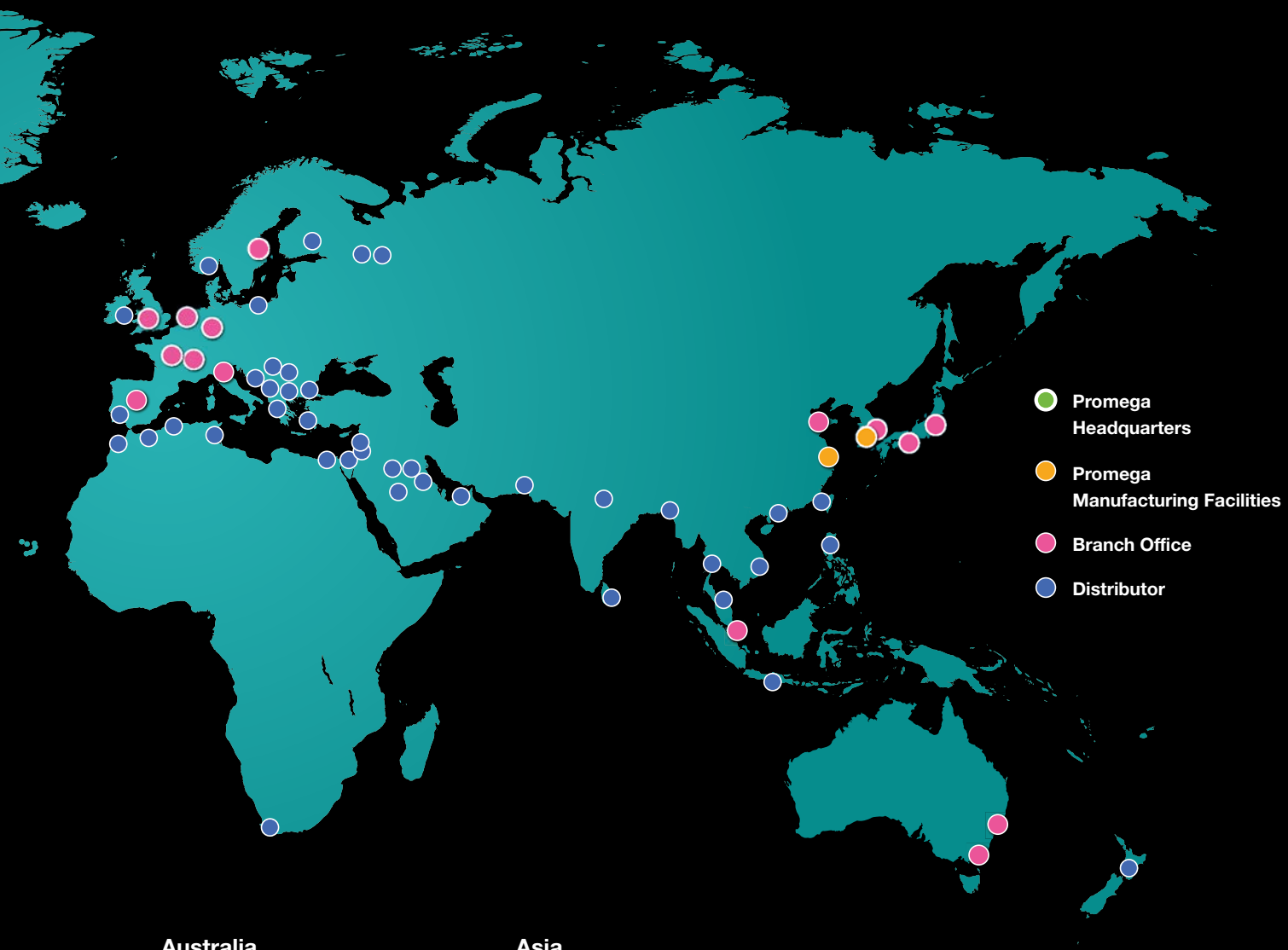
Poland
Tel: +48 22 531 0667
Fax: +48 22 531 0669
E-mail: pl_custserv@promega.com

Belgium/Luxembourg/
The Netherlands
Tel: +31 71 532 42 44
Fax: +31 71 532 49 07
E-mail: benelux@promega.com

Spain
Tel: +34 902 538 200
Fax:+34 902 538 300
E-mail: esp_custserv@promega.com

Denmark, Estonia, Finland,
Iceland, Norway, Sweden
Tel: +46 8 452 2450
Fax: +46 8 452 2455
E-mail: sweorder@promega.com

United Kingdom
Tel: +44 23 8076 0225
Fax: +44 23 8076 7014
Free Phone: 0800 378994
E-mail: ukcustserve@promega.com



Australia

Australia
Tel: 02 8338 3800
Fax: 02 8338 3855
Freecall: 1800 225123
Freefax: 1800 626 017
E-mail: auscustserv@promega.com

Asia

China
Tel: +86 10 5825 6268
Fax: +86 10 5825 6160
Toll-Free: 800 810 8133
E-mail: info@promega.com.cn

Japan
Tel: 03 3669 7981
Fax: 03 3669 7982
E-mail: jpmktg@jp.promega.com

Korea
Tel: +82 2158 83718
Fax: +82 2262 85418
E-mail: CustServiceKR@promega.com

Pacific Asia Region, Singapore
Tel: +65 6513 3450
Fax: +65 6773 5210
E-mail: sg_custserv@promega.com

India
Tel: +91 11 43005814/15/16/17
Fax: +91 11 41035028
E-mail: ind_custserv@promega.com

For more information about Promega Branch Offices and Distributors visit:

www.promega.com/support/worldwide-contacts