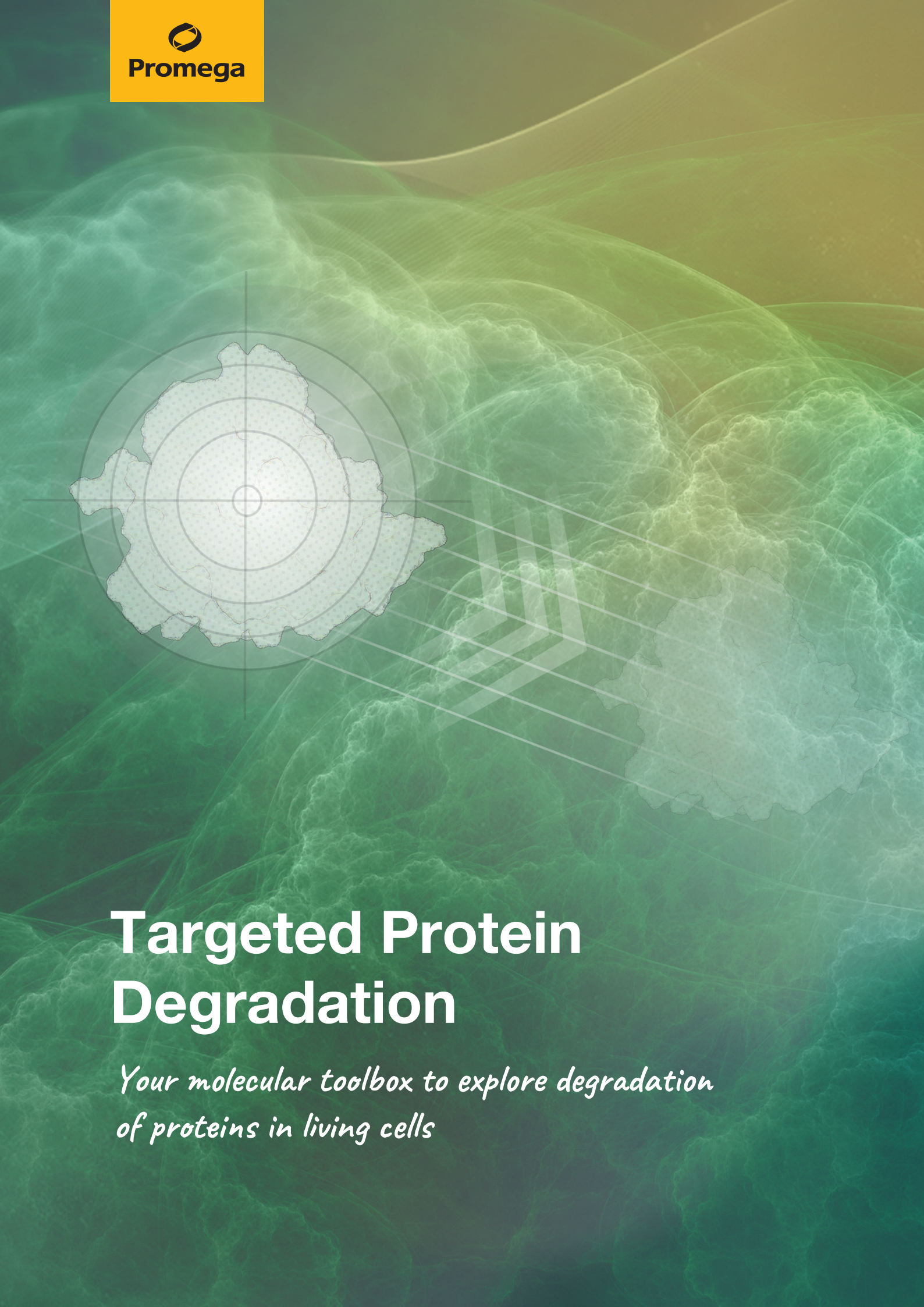
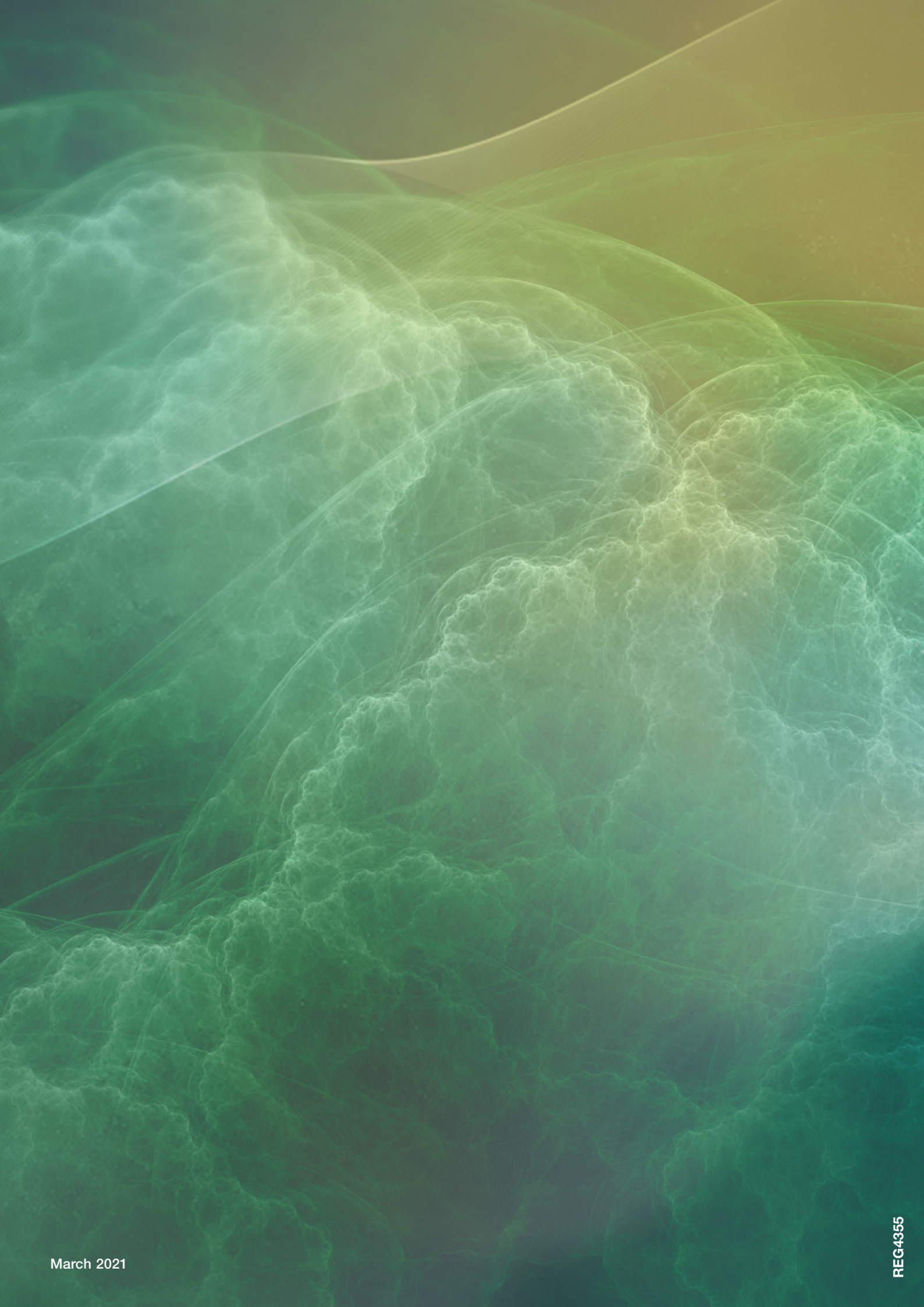
Promega



Targeted Protein Degradation

Your molecular toolbox to explore degradation of proteins in living cells



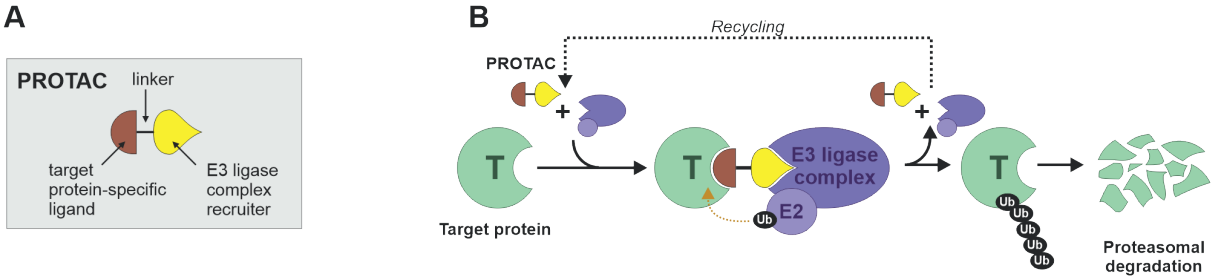
March 2021

REG4355

(Targeted) Protein Degradation

Protein degradation is a key mechanism involved in maintaining cellular proteostasis. Impairment of lysosomal and/or proteasomal removal of non-functional proteins can lead to the formation of aggregates that are causally involved in the genesis of various diseases, including *Morbus Parkinson* and *Morbus Alzheimer*.
On the other hand, distinctive degradation of target proteins by hijacking the cellular degradation machinery holds great promise as a novel therapeutic strategy to treat diseases such as cancer, autoimmune, and neurological disorders. These degrader drugs may provide several benefits over to conventional small molecule-based therapies, e.g. expansion of the "druggable" proteome, prolonged pharmacokinetics, and a catalytic mode-of-action could enable the use of lower systemic concentrations. Molecular glues and proteolysis targeting chimeras (PROTACs) are the most prominent representatives of this novel drug class of small molecule degraders (e.g. LYTACs, PHOTACs, PROTACs, molecular glues, AUTACs, hydrophobic tags) that have been developed to date.

PROTAC Structure and Mechanism of Action

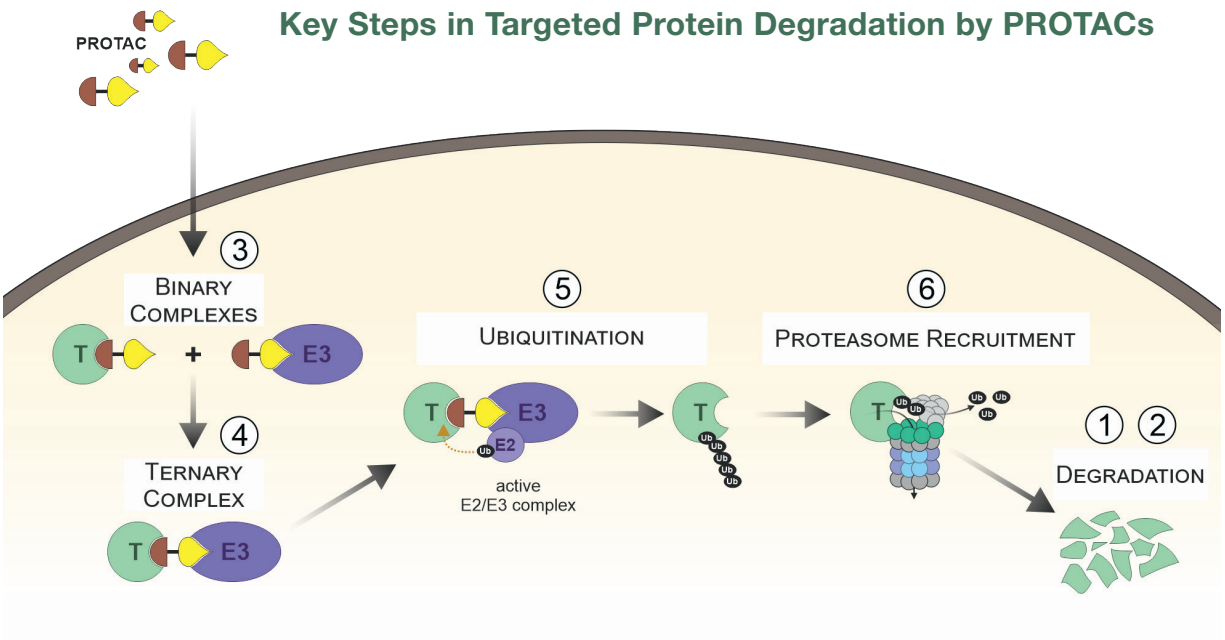


PROTACs are heterobifunctional molecules composed of two ligand domains coupled by a linker of variable length. One ligand binds to the target protein (T) while the other facilitates the recruitment of an E3 ligase complex **(A)**. PROTACs act by linking a target protein (T) to an E2/E3 ligase complex. Spatial proximity enables polyubiquitination and subsequent proteasomal degradation of the target protein while the PROTAC recycles **(B)**.

Elementary Questions in PROTAC Development

1. Is the target protein efficiently degraded?
2. Does degradation lead to the desired cellular phenotype?
3. Is the PROTAC permeable and does it bind to either target or E3 ligase?
4. Does the PROTAC facilitate the formation of a ternary complex?
5. Is the target protein efficiently ubiquitinated?
6. Is the ubiquitinated target protein recruited to the cellular proteasome?

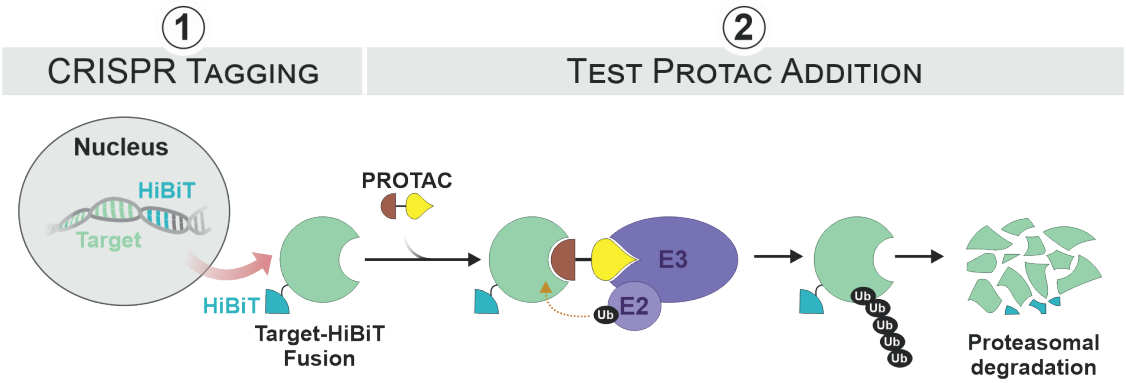
Key Steps in Targeted Protein Degradation by PROTACs



Degradation of Target Protein

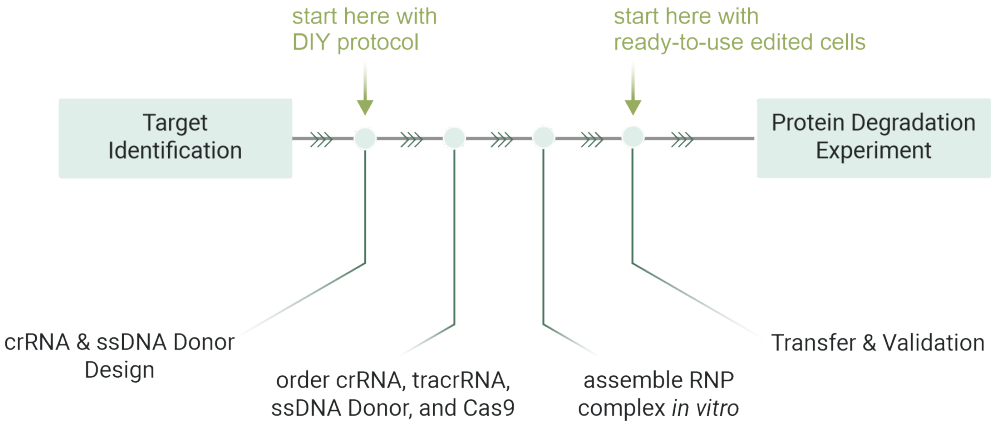
The degradation of a given target protein of interest is the desired outcome when using PROTACs. In the past immuno-based detection as well as mass spectrometry has been employed intensively to verify loss of target protein. While these strategies allow for the detection of endogenous proteins, they both involve cumbersome multistep processes. Furthermore, the fact that these are lytic endpoint assays, limits their ability to easily capture multiphasic degradation profiles. While tagging with autofluorescent proteins supports monitoring target loss in real-time their high molecular weight complicates targeted genomic insertion and renders it to be less efficient. Endogenous expression under the control of the gene's native promoter, however, has proven to be indispensable to obtain physiologically relevant degradation data. The bioluminescent peptide tag HiBiT ("*High BiT*") meets all these requirements. HiBiT is an 11 aa peptide subunit derived from NanoLuc® luciferase, which can be detected by binding the complementary LgBiT ("*Large BiT*") subunit with extremely high affinity (K_D = 700 pM). Together these subunits reconstitute the functional NanoBiT® luciferase that can be easily detected via bioluminescence. HiBiT-tagged proteins can therefore be quantified by providing LgBiT and the luciferase substrate furimazine.

Assay Workflow



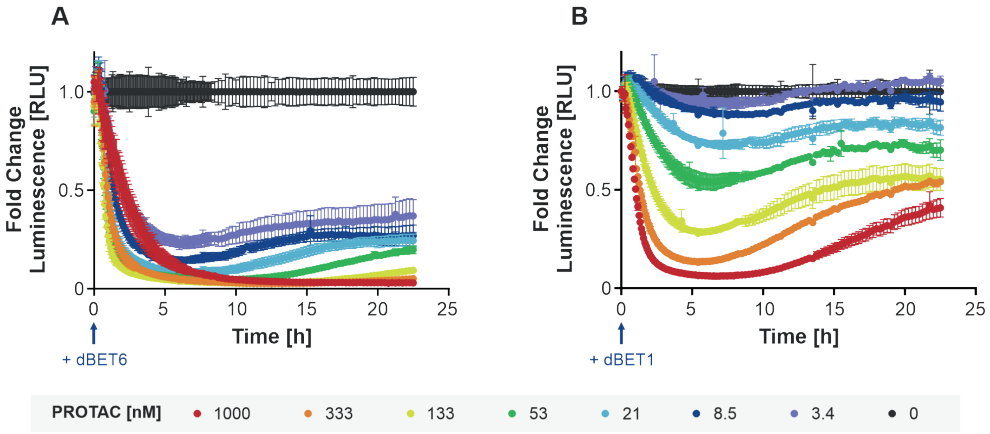
The HiBiT-encoding sequence is inserted into the desired genomic locus using CRISPR/Cas9 technology **(1)**. Following PROTAC addition, the degradation/recovery of the recombinant HiBiT fusion protein can be determined either in a lytic endpoint assay or real-time kinetic experiment in live cells **(2)**.

Your Routes to Endogenously Tagged HiBiT Fusions



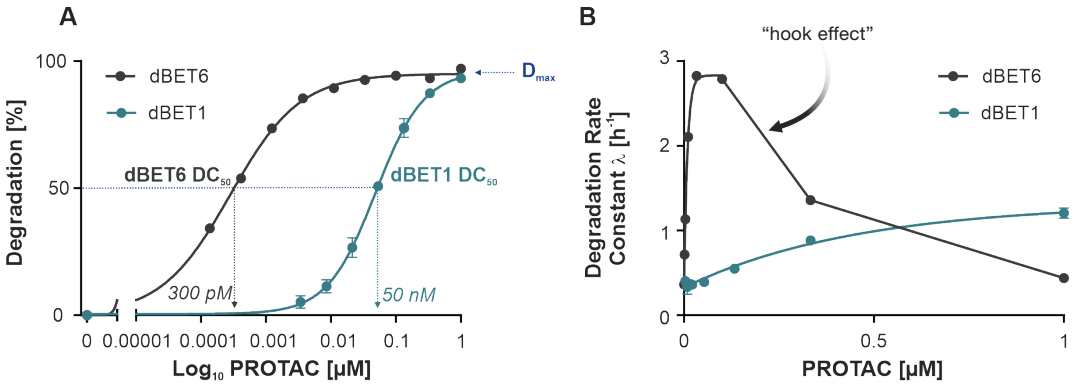
Either *do-it-yourself* with a cloning-free protocol for rapid success or choose from our growing collection of assay-ready CRISPR-edited cell lines for maximum convenience.

Quantification of Protein Loss in Real-time Upon PROTAC Treatment



CRISPR-mediated tagging of BET family member BRD4 with HiBiT in cells stably expressing the LgBiT subunit enables the monitoring of targeted endogenous protein degradation in real-time. Before the addition of PROTACs dBET6 **(A)** and dBET1 **(B)** at time zero, cells were pre-equilibrated with the extended Nano-Glo® Endurazine™ Live-Cell Substrate. The luminescent signal was recorded over a period of 24 hours to determine HiBiT-BRD4 degradation and recovery.

Calculation of Quantitative Parameters from Real-time Degradation Profiles



Recording of real-time protein degradation and recovery profiles allows for determination of quantitative degradation parameters, i.e. percent degradation, half-maximal degradation concentration (DC_{50}), maximal level of degradation (D_{max}) **(A)**, and degradation rate **(B)**. These can be used for rank ordering of compounds. At high dBET6 concentrations, the degradation rate decreases due to hindered formation of ternary complexes (target protein:PROTAC:E3 ligase) also known as "hook effect".

Product Box

LgBiT Stable Cell Lines
- HEK293
- Jurkat
- HeLa

LgBiT Expression Vector

Ready-to-use CRISPR/HiBiT Cell Lines

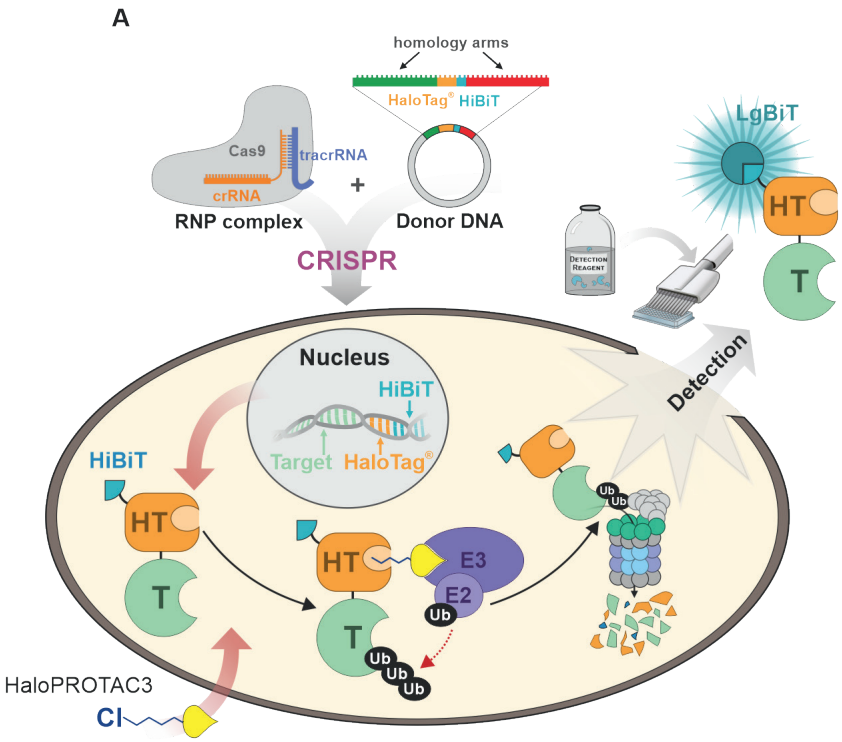
Detection Reagents
- Nano-Glo® HiBiT Lytic Detection System
- Nano-Glo® HiBiT Extracellular Detection System
- Nano-Glo® HiBiT Blotting System
- Nano-Glo® Live-Cell Assay System (0 – 2 h)
- Nano-Glo® Vivazine™ Substrate (2 – 24 h)
- Nano-Glo® Endurazine™ Substrate (2 – 72 h)

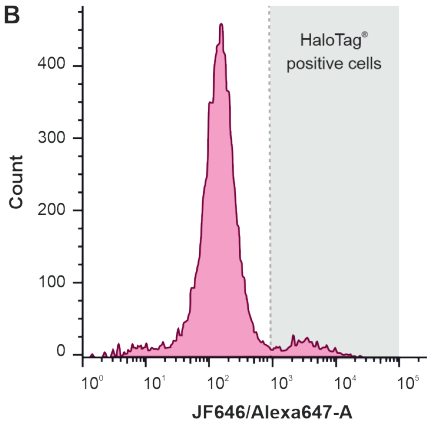
see page 13 for more product details

Degradation Phenotype

The identification of suitable targets whose knock-down is accompanied by the desired phenotypic consequences usually precedes any efforts for PROTAC development. HaloPROTAC3 offers an easy way to evaluated degradation phenotypes by mediating targeted degradation of HaloTag® (HT) fusion proteins.

Assay Workflow

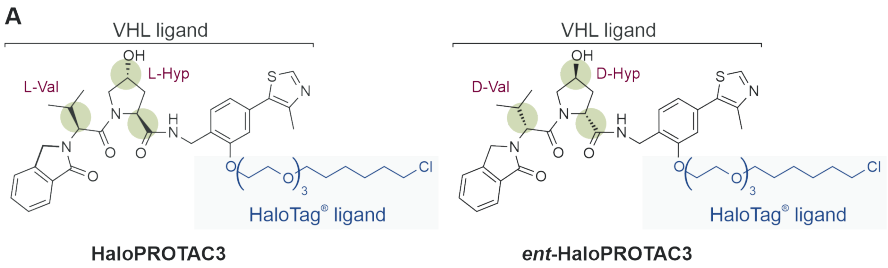


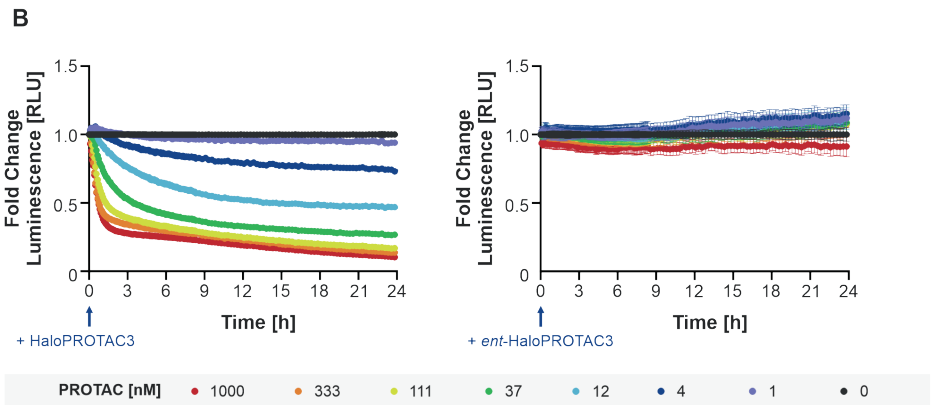


The gene of interest is tagged endogenously with tandem HiBiT-HaloTag® using CRISPR **(A)**. The presence of the recombinant target (T) protein in the edited cell pool can be easily verified using either luminescent HiBiT detection reagents or by staining with fluorescent Janelia HaloTag® ligands. Furthermore, these ligands can promote isolation of positive clones through Fluorescence Activated Cell Sorting (FACS) **(B)**. Treatment of cells with HaloPROTAC3 mediates targeted degradation of HiBiT-HaloTag®-target protein that can be determined using the HiBiT moiety.

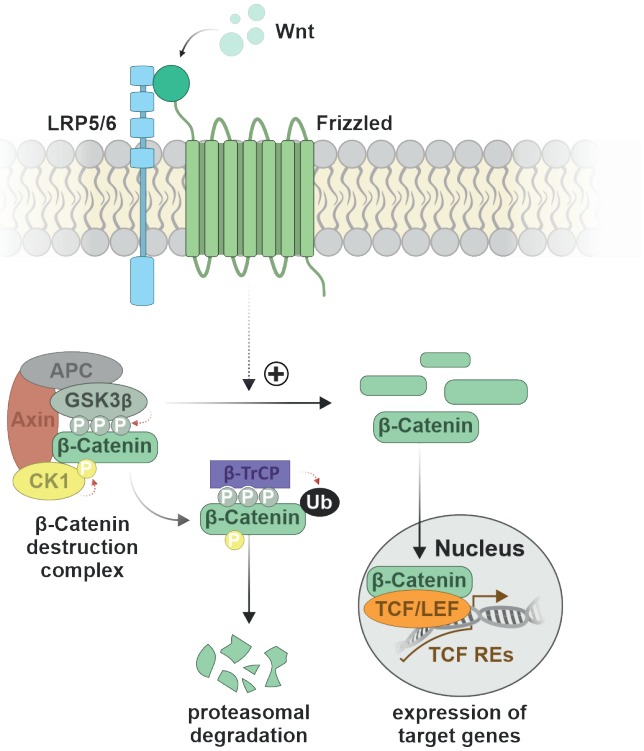
Confirm Degradation Through VHL Engagement and a PROTAC Mechanism

The enantiomeric compound *ent*-HaloPROTAC3 can be used along with HaloPROTAC3 to confirm that degradation of a HaloTag® fusion protein is mediated through a VHL engagement and a PROTAC mechanism. The *ent*-HaloPROTAC3 consists of the same molecular weight and general structure. The D-hydroxyproline (D-Hyp) and D-valine (D-Val) residue modifications, however, significantly disrupt binding to VHL **(A)**. CRISPR-edited HEK293 cells, coexpressing a HiBiT-HaloTag®-BRD4 fusion and the LgBiT subunit were treated with varying concentrations of either HaloPROTAC3 variant. The protein level of recombinant BRD4 was monitored in realtime. While HaloPRTOAC3 yields a concentration-dependent loss of target protein, no degradation was detected in presence of *ent*-HaloPROTAC3 **(B)**.



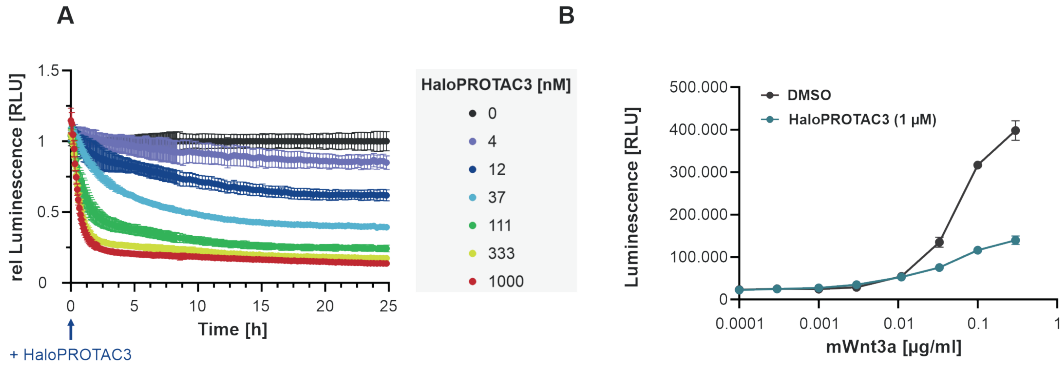


The Wnt/β-Catenin Model System



β-Catenin is a key component of the canonical Wnt signaling pathway. Through this clinically relevant pathway, cells can integrate extracellular signals, to control various aspects of embryonic development. Under nonstimulated conditions, β-Catenin is bound and phosphorylated by the β-Catenin destruction complex. This enables the recruitment of the E3 ligase β-TrCP leads to polyubiquitination and proteasomal degradation of β-Catenin. The activation of the pathway through binding of the Wnt ligand to the membrane receptor Frizzled promotes the cytosolic accumulation of β-Catenin and followed by its nuclear translocation. Here it serves as a transcriptional co-activator that initiates transcription of Wnt-responsive genes by binding to T-cell factor/ lymphoid enhancer factor (TCF/LEF) transcription factors.

Linking Target Loss with Phenotypic Consequences



The transcriptional co-activator β-catenin, a key constituent of the Wnt signaling pathway, was endogenously tagged with HiBiT-HaloTag®. **(A)** Upon treatment with HaloPROTAC3, its time- and dose-dependent degradation was verified. **(B)** The resulting repression of mWnt3a-induced β-catenin/TCF-mediated transcription was determined using a firefly-based gene reporter plasmid with TCF response elements (RE).

Product Box

HaloPROTAC3
***ent*-HaloPROTAC3** (Negative Control)
HiBiT-HaloTag® CRISPR Donor Vector (N-terminal)
HaloTag®-VS-HiBiT CRISPR Donor Vector (C-terminal)
HaloTag® CRISPR Donor Vector (N-terminal)
HaloTag® CRISPR Donor Vector (C-terminal)
Janelia Fluor® 549 HaloTag® Ligand
Janelia Fluor® 646 HaloTag® Ligand
LgBiT Expression Vector

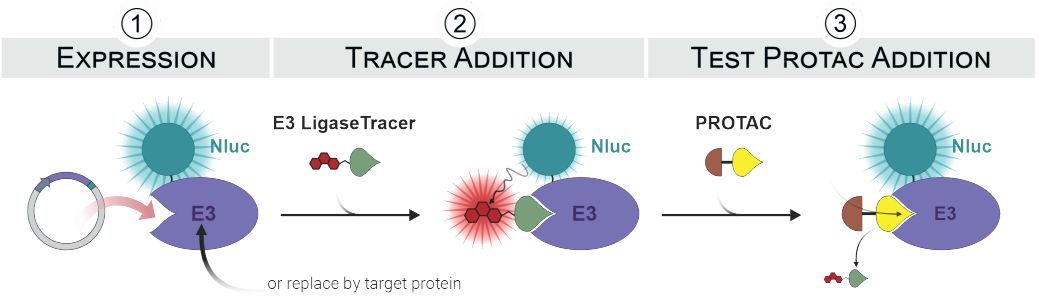
Detection Reagents
- Nano-Glo® HiBiT Lytic Detection System
- Nano-Glo® HiBiT Blotting System
- Nano-Glo® Live-Cell Assay System (0 – 2 h)
- Nano-Glo® Vivazine™ Substrate (2 – 24 h)
- Nano-Glo® Endurazine™ Substrate (2 – 72 h)

see page 13 for more product details

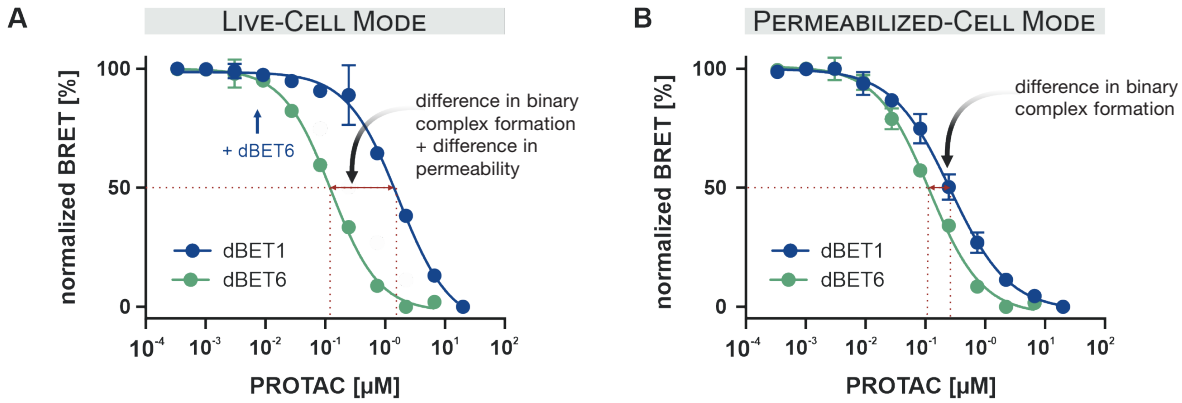
PROTAC Permeability and Binary Complex Formation

The ability of a PROTAC to traverse the cellular membrane is a prerequisite for executing its molecular function. NanoBRET™ Target Engagement (TE) is a powerful technology to investigate protein:ligand binding events in live cells. Here, the protein of interest (POI) – either the target protein (T) or the desired E3 ligase – is ectopically expressed as a fusion to NanoLuc® luciferase followed by addition of a cell-permeable fluorescent tracer molecule with known affinity to the POI. Tracer binding yields spatial proximity of luciferase (donor) and fluorophore (acceptor) enabling bioluminescent resonance energy transfer (BRET) to occur. PROTAC binding can be determined by a decrease in BRET due to competitive displacement of the tracer. Besides studying binary complex formation, NanoBRET™ TE allows to assess PROTAC permeability.

Assay Workflow



Quantitate Intracellular Affinity and Assess Compound Permeability



The NanoBRET™ TE CRBN assay was used to quantitate intracellular affinity of the PROTACs dBET1 and dBET6 using the live-cell mode **(A)**. Differences in affinity determined in live-cell mode can be due to a difference in intrinsic binary complex affinity and/or a difference in compound permeability. The permeabilized-cell mode was used to determine intrinsic binary complex affinity **(B)**. Using both live and permeabilized mode data allows determination of compound intracellular availability, a measure of compound permeability.

Product Box

NanoBRET™ TE Intracellular E3 Ligase Assays
- Cereblon (CRBN)
- Von Hippel-Lindau disease tumor suppressor (VHL)
- E3 ubiquitin-protein ligase Mdm2 (MDM2)
- Inhibitor of apoptosis proteins (IAP), i.e. cIAP1 and XIAP

Detection Reagents
- Intracellular TE Nano-Glo® Substrate/Inhibitor (0 – 2 h)
- Intracellular TE Nano-Glo® Vivazine™/Inhibitor (2 – 24 h)

NanoBRET™ TE Expression Vectors
- DDB1 (for co-expression in CRBN assay)

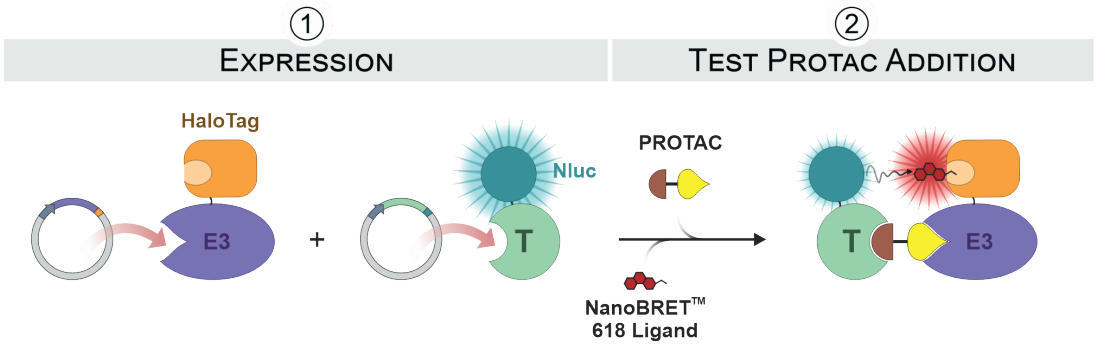
NanoLuc® Fusion Vectors & Validated NanoBRET™ TE Assays
- Kinases
- BET-family proteins
- HDACs
- Heat shock protein 90 (Hsp90)
- NLR family pyrin domain containing 3 (NLRP3)

see page 14 for more product details

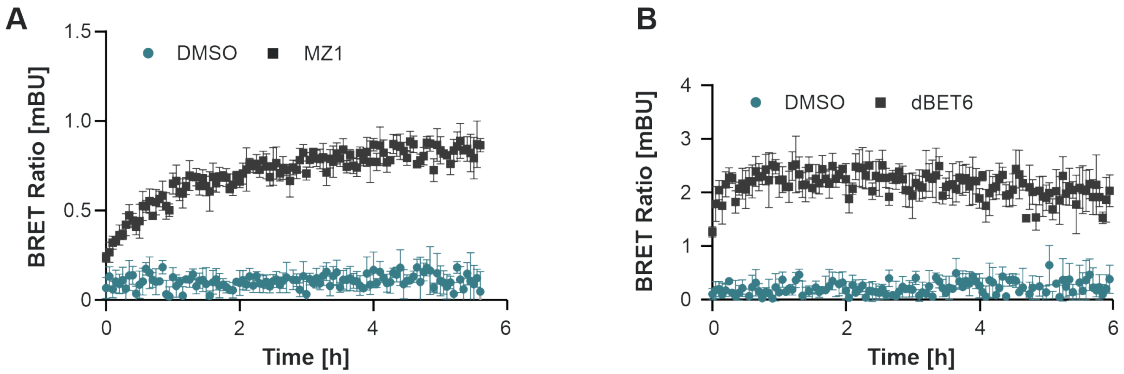
Ternary Complex Formation

PROTAC-mediated linkage of target protein and an E3 ligase complex, i.e. the formation of a ternary complex, resembles a fundamental step to achieve targeted protein degradation. The dynamic nature of protein:protein interactions (PPI), i.e. their association as well as dissociation, can be reliably studied in live cells using NanoBRET™ Target PPI Technology. Therefore, the target protein (T) is ectopically expressed as a fusion to NanoLuc® luciferase along with a E3 ligase:HaloTag® fusion. Successful ternary complex formation leads to spatial proximity of luciferase (donor) and the HaloTag-E3 ligase (acceptor) labeled with the cell-permeable fluorescent NanoBRET™ 618 HaloTag® Ligand enabling bioluminescent resonance energy transfer (BRET) to occur. Association, as well as dissociation of this complex, can thus be followed by an increase or decrease in BRET respectively. This assay can be optionally performed with endogenously tagged target-donor fusions.

Assay Workflow



Monitor Ternary Complex Formation in Real-Time



Determination of ternary complex formation between endogenously tagged HiBiT-BRD4 and ectopically expressed HaloTag®-VHL **(A)** and HaloTag®-CRBN **(B)** upon treatment with the PROTACs MZ-1 (1 µM) or dBET6 (0.1 µM) respectively. Data were recorded over 6 hours using the NanoBRET™ Kinetic Detection System.

Product Box

NanoBRET™ CRBN Ternary Complex Starter Kit
HaloTag®-CRBN Fusion Vector
HaloTag®-CRBN HEK293 Cell Line
NanoBRET™ VHL Ternary Complex Starter Kit
HaloTag®-VHL Fusion Vector
HaloTag®-VHL HEK293 Cell Line
HaloTag® Control Vector (Negative Control)
NanoLuc®-BRD4 FL Fusion Vector (Positive Control)

Detection Reagents
- NanoBRET™ Nano-Glo® Detection System (0 – 2 h)
- NanoBRET™ Nano-Glo® Kinetic Detection System (2 – 24 h)

Is your target a kinase, BET-family or HDAC protein?

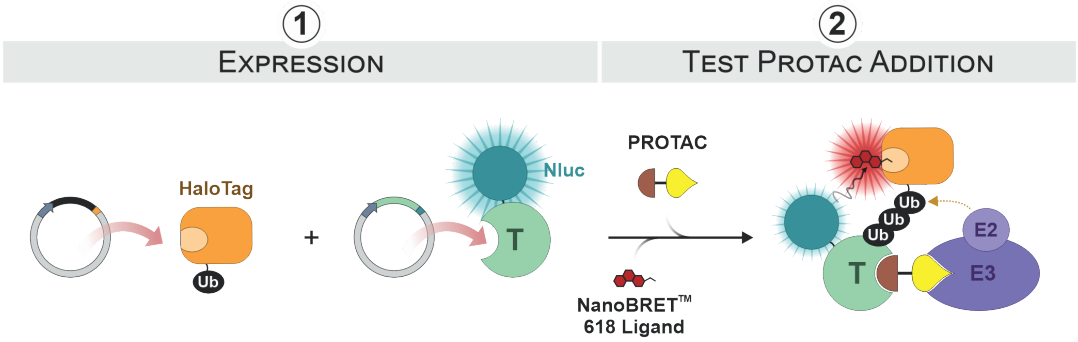
Refer to our huge selection of pre-cloned NanoLuc® fusion vectors.

see page 19 for more product details

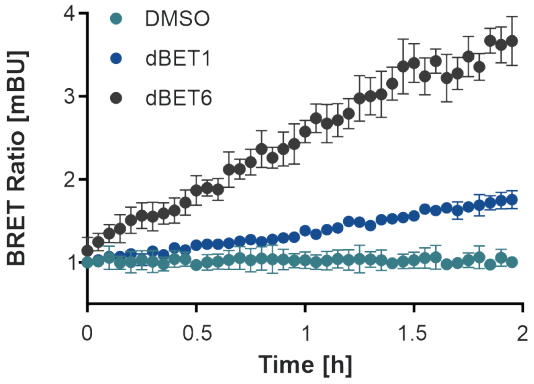
Target Protein Ubiquitination

Proteins targeted for degradation via the ubiquitin-proteasome system (UPS) require efficient ubiquitin conjugation prior to proteasome trafficking. Ubiquitination on any given target can vary in levels, mono- and poly-ubiquitination, and be mediated through a variety of amino acid linkages. Using NanoBRET™ all types of ubiquitination, in terms of extent and linkage, can be broadly determined. Therefore, a recombinant HaloTag®-ubiquitin and the target protein (T) as NanoLuc® (Nluc) fusion are ectopically co-expressed and the BRET signal is measured upon PROTAC treatment. This assay can be optionally performed with endogenously tagged target-donor fusions.

Assay Workflow



Determine PROTAC Potency to Induce Target Ubiquitination



Ubiquitination of ectopically expressed HiBiT-BRD4 following PROTAC treatment – dBET1 (1 µM) and dBET6 (0.1 µM) – as determined by NanoBRET™. Ubiquitination signal increases faster and to a greater extent with dBET6 compared to dBET1 despite a 10-fold lower concentration applied.

Product Box

NanoBRET™ Ubiquitination Starter Kit

HaloTag®-Ubiquitin Fusion Vector

HaloTag® Control Vector (Negative Control)

NanoLuc®-BRD4 FL Fusion Vector (Postive Control)

Detection Reagents

- NanoBRET™ Nano-Glo® Detection System (0 – 2 h)
- NanoBRET™ Nano-Glo® Kinetic Detection System (2 – 24 h)

Is your target a kinase, BET-family or HDAC protein?

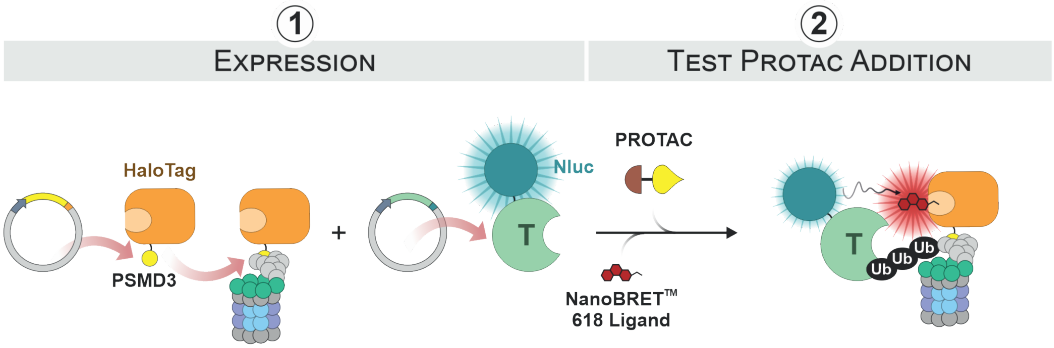
Refer to our huge selection of pre-cloned NanoLuc® fusion vectors.

see page 19 for more product details

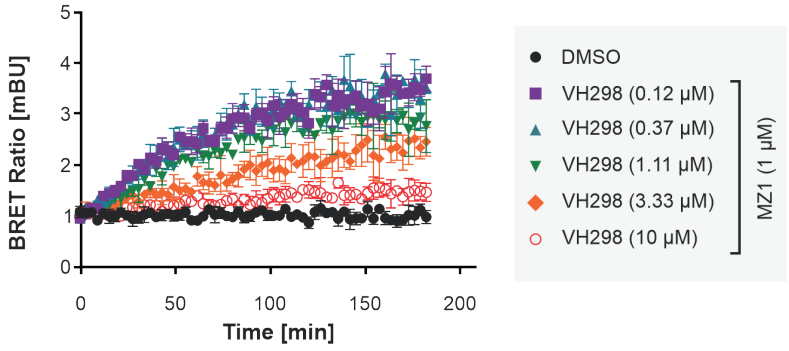
Proteasomal Recruitment

Most eukaryotic proteins are degraded by recruiting and processing mediated by the 26S proteasome following polyubiquitination. This process can be monitored in live cells using NanoBRET™. Because of the dynamics of protein stabilization or degradation, the optimal NanoBRET™ assay configuration is with the target protein (T) fused to NanoLuc® as the luminescent donor and the 26S proteasomal component as the fluorescent acceptor. This means you can monitor target protein levels while simultaneously observing proteasomal recruitment, made possible due to the luminescent/fluorescent ratio in the NanoBRET™ assay. Amongst the many proteasomal subunits, PSMD3 (26S proteasome regulatory subunit 3) was identified to be the optimal proteasomal protein for this general assay when N-terminally fused to HaloTag®. This acceptor fusion can be labeled with the HaloTag® NanoBRET™ 618 Ligand to be a fluorescent acceptor. This assay can be optionally performed with endogenously tagged target-donor fusions.

Assay Workflow



Interrogate Proteasomal Recruitment of Polyubiquitinated Target Proteins



Time course analysis of HiBiT-BRD4 recruitment to the 26S proteasome following treatment with the PROTAC MZ-1 (1 µM). Co-treatment with the parental compound VH298 (i.e. the VHL recognition domain of MZ-1) confirms assay specificity. The BRET signal decreases with increasing concentrations of VH298 due to competitive displacement of MZ-1.

Product Box

NanoBRET™ Proteasomal Recruitment Starter Kit

HaloTag®-PSMD3 Fusion Vector

HaloTag® Control Vector (Negative Control)

NanoLuc®-BRD4 FL Fusion Vector (Positive Control)

Detection Reagents

- NanoBRET™ Nano-Glo® Detection System (0 – 2 h)
- NanoBRET™ Nano-Glo® Kinetic Detection System (2 – 24 h)

Is your target a kinase, BET-family or HDAC protein?

Refer to our huge selection of pre-cloned NanoLuc® fusion vectors.

see page 20 for more product details

GloMax® Detection Systems

A versatile, reliable, and intuitive lab companion to support your research

GloMax® Discover is an advanced multimode plate reader designed to provide optimal performance for Promega reagents with high-performance luminescence, fluorescence, UV-visible absorbance, BRET and FRET, two-color filtered luminescence, and kinetic measurement capabilities. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

One instrument for numerous applications:

- Reporter gene assays
- Cell viability, cytotoxicity, and apoptosis assays
- Kinetic measurements
- Multiplexing
- Oxidative stress and cell metabolism
- ELISA
- BRET/FRET analysis
- Lumit™ Immunoassays

GloMax DISCOVER

A high-performance, easy-to-use multimode plate reader for luminescence, fluorescence, absorbance, BRET and FRET applications

For more information visit: www.promega.com/glomax-comparison

Ready-to-use CRISPR/HiBiT Cells

Browse our growing selection of ready-to-useHiBiT/CRISPR cells:

www.promega.com/hibit-crispr-knock-in

Product Order Information

HiBiT Protein Degradation Assays (page 4–7)

Nano-Glo® HiBiT Detection Reagents

	Cat. #	Quantity
Nano-Glo® HiBiT Lytic Detection System • Nano-Glo® HiBiT Lytic Substrate • Nano-Glo® HiBiT Lytic Buffer • LgBiT Protein	N3030 N3040 N3050	10 ml 100 ml 10 x 100 ml
Nano-Glo® HiBiT Extracellular Detection System • Nano-Glo® HiBiT Extracellular Substrate • Nano-Glo® HiBiT Extracellular Buffer • LgBiT Protein	N2420 N2421 N2422	10 ml 100 ml 10 x 100 ml
Nano-Glo® HiBiT Blotting System	N2410	100 ml
Nano-Glo® Live Cell Assay System (0 – 2 h) • Nano-Glo® Live Cell Substrate • Nano-Glo® LCS Dilution Buffer	N2011 N2012 N2013	10 ml 100 ml 10 x 100 ml
Nano-Glo® Vivazine™ Substrate (2 – 24 h)	N2580 N2581 N2582	0.1 ml 1 ml 10 ml
Nano-Glo® Endurazine™ Substrate (2 – 72 h)	N2570 N2571 N2572	0.1 ml 1 ml 10 ml

Expression of Intracellular LgBiT Subunit

	Cat. #	Quantity
LgBiT Expression Vector	N2681	20 µg
HEK293 LgBiT Stable Cell Line	N2627	2 vials
Jurkat LgBiT Stable Cell Line	CS1956D07	2 vials
HeLa LgBiT Stable Cell Line	CS1956D05	2 vials

For additional LgBiT expressing cell lines please inquire.

Degradation of HaloTag® HiBiT Fusion Proteins

	Cat. #	Quantity
HaloPROTAC3, 2.5 mM	GA3110	20 µl
ent-HaloPROTAC3, 2.5 mM (Negative Control)	GA4110	20 µl
NanoBRET™ Positive Control Vector (Positive Control)	N1581	20 µg
HaloTag®-HiBiT Vector [CAG / Blast] (Positive Control)	CS1956B17	20 µg
HiBiT-HaloTag® CRISPR Donor Vector (N-terminal)	CS3023278	20 µg
HaloTag®-VS-HiBiT CRISPR Donor Vector (C-terminal)	CS3023277	20 µg
HaloTag® CRISPR Donor Vector (N-terminal)	please enquire	20 µg
HaloTag® CRISPR Donor Vector (C-terminal)	please enquire	20 µg
Janelia Fluor® 549 HaloTag® Ligand	GA1110 GA1111	5 µg 3 x 5 µg
Janelia Fluor® 646 HaloTag® Ligand	GA1120 GA1121	5 µg 3 x 5 µg

Product Order Information

NanoBRET™ Target Engagement (TE) (page 8)

NanoBRET™ TE Intracellular E3 Ligase Assays

	Cat. #	Quantity
NanoBRET™ TE Intracellular E3 Ligase Assay, CRBN • NanoLuc®-CRBN Fusion Vector * • NanoBRET™ TE Tracer CRBN, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • DDB1 Expression Vector *	N2910 N2911 N2912	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular E3 Ligase Assay, VHL • VHL-NanoLuc® Fusion Vector * • NanoBRET™ TE Tracer VHL, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2930 N2931 N2932	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular E3 Ligase Assay, IAP • NanoLuc®-XIAP Fusion Vector * • NanoBRET™ TE Tracer IAP, 100 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	CS1810C431 CS1810C484	1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular E3 Ligase Assay, MDM2 • NanoLuc®-MDM2 Fusion Vector * • NanoBRET™ TE Tracer MDM2 • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	CS (TBD) CS (TBD	1,000 assays (96-well) 10,000 assays (96-well)

* not contained in 10,000 assay kit

Need technical assistance?

please contact our technical service:

www.promega.com/support/tech-support

Product Order Information

NanoBRET™ TE Kinase Assays

	Cat. #	Quantity
NanoBRET™ TE Intracellular Kinase Assay, K-3 • NanoLuc®-PIK3CA Fusion Vector * • NanoBRET™ Tracer K-3, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • PIK3R1 Expression Vector * • Transfection Carrier DNA *	N2600 N2601 N2810	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-4 • DDR1-NanoLuc® Fusion Vector * • NanoBRET™ Tracer K-4, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2520 N2521 N2540	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-5 • BTK-NanoLuc® Fusion Vector * • NanoBRET™ Tracer K-5, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2500 N2501 N2530	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-8 • NanoLuc®-GSK3B Fusion Vector * • NanoBRET™ Tracer K-8, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2620 N2621 N2820	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-9 • NanoLuc®-PLK2 Fusion Vector * • NanoBRET™ Tracer K-9, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2630 N2631 N2830	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-10 • LIMK1-NanoLuc® Fusion Vector * • NanoBRET™ Tracer K-10, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2640 N2641 N2840	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular Kinase Assay, K-11 • ACVR1-NanoLuc® Fusion Vector * • NanoBRET™ Tracer K-11, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2650 N2651 N2850	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)

* not contained in 10,000 assay kit

Product Order Information

NanoBRET™ TE Intracellular HDAC Assays

	Cat. #	Quantity
NanoBRET™ TE Intracellular HDAC Assay • NanoLuc®-HDAC6 FL Fusion Vector * • NanoBRET™ Intracellular TE HDAC Tracer, 100 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2080 N2081 N2090	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular HDAC Complete Kit • NanoBRET™ TE Intracellular HDAC Assay • NanoBRET™ TE HDAC DNA Bundle	N2170	1,000 assays (96-well)

* not contained in 10,000 assay kit

NanoBRET™ TE Intracellular BET BRD Assays

	Cat. #	Quantity
NanoBRET™ TE Intracellular BET BRD Assay • NanoLuc®-BRD4 FL Fusion Vector * • NanoBRET™ Intracellular TE BET BRD Tracer, 100 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	N2130 N2131 N2140	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ TE Intracellular BET BRD Complete Kit • NanoBRET™ TE Intracellular BET BRD Assay • NanoBRET™ TE BET BRD DNA Bundle	N2180	1,000 assays (96-well)
NanoBRET™ TE Intracellular BRD Assay-02 • NanoLuc®-BRD4 FL Fusion Vector • NanoBRET™ BRD Tracer-02, 400 µM • Intracellular TE Nano-Glo® Substrate/Inhibitor • Tracer Dilution Buffer	CS1810C21	1,000 assays (96-well)

* not contained in 10,000 assay kit

NanoBRET™ TE Assays for Other Targets

	Cat. #	Quantity
NanoBRET™ TE HSP90 Assay • NanoBRET™ TE Tracer HSP90, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor	CS1810C458	10,000 assays (96-well)
NanoBRET™ TE NLRP3 Assay • NLRP3-NanoLuc® Fusion Vector * • NanoBRET™ TE Tracer NLRP3, 400 µM • Tracer Dilution Buffer • Intracellular TE Nano-Glo® Substrate/Inhibitor • Transfection Carrier DNA *	CS1810C523	1,000 assays (96-well)

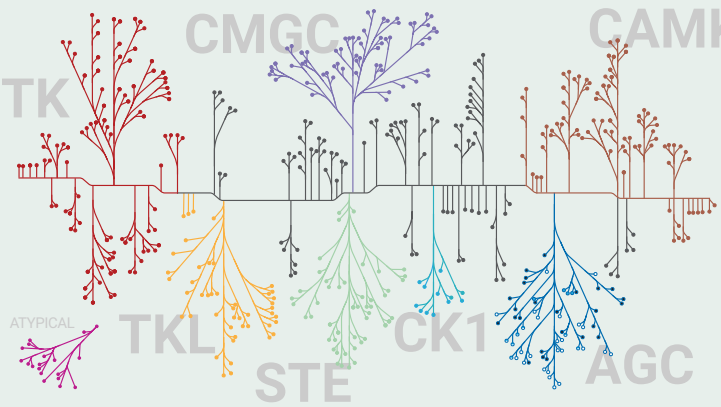
Product Order Information

NanoBRET™ TE NanoLuc® Fusion Vectors

	Cat. #	Quantity
E3 Ligases		
NanoLuc®-CRBN Fusion Vector	N2741	20 µg
VHL-NanoLuc® Fusion Vector	N2751	20 µg
NanoLuc®-cIAP1 Fusion Vector	CS1810C435	20 µg
NanoLuc®-XIAP Fusion Vector	CS1810C110	20 µg
Targets		
HDAC8-NanoLuc® Fusion Vector	CS181001F	20 µg
NanoLuc®-HSP90AB1 Fusion Vector	CS1810C461	20 µg
NLRP3-NanoLuc® Fusion Vector	CS1810C450	20 µg

	Cat. #	Quantity
NanoBRET™ TE HDAC DNA Bundle • HDAC1-NanoLuc® FL Fusion Vector • HDAC2-NanoLuc® FL Fusion Vector • HDAC3-NanoLuc® FL Fusion Vector • NanoLuc®-HDAC6 FL Fusion Vector • NanoLuc®-HDAC6 (CD2) Fusion Vector • HDAC10-NanoLuc® FL Fusion Vector	N2120	20 µg each
NanoBRET™ TE BET BRD DNA Bundle • NanoLuc®-BRD2 FL Fusion Vector • NanoLuc®-BRD2 BD1 Fusion Vector • NanoLuc®-BRD2 BD2 Fusion Vector • NanoLuc®-BRD3 FL Fusion Vector • NanoLuc®-BRD4 FL Fusion Vector • NanoLuc®-BRD4 BD1 Fusion Vector • NanoLuc®-BRD4 BD2 Fusion Vector • NanoLuc®-BRDT FL Fusion Vector	N2150	20 µg each

NanoLuc® Kinase Fusion Vectors



Browse our growing selection of NanoLuc® Kinase Fusion Vectors:

- 263 full length kinases
- 79 kinase mutants
- 4 kinase domains

www.promega.com/kinasevectors

Product Order Information

NanoBRET™ TE Expression Vectors

	Cat. #	Quantity
DDB1 Expression Vector	N2761	3 x 20 µg

Additional expression vectors for co-expression of kinase regulatory proteins (e.g. cyclins) are available.

NanoBRET™ TE Stable NanoLuc® Cell Lines

	Cat. #	Quantity
NanoLuc®-CRBN HEK293 Cell Line	CS1810C398	2 vials
VHL-NanoLuc® HEK293 Cell Line	CS1810C393	2 vials
NLRP3-NanoLuc HEK293 Stable Cell Line	CS1810C494	2 vials
BRD4 NanoBRET™ TE HEK293 Cell Line	CS1810C09	2 vials
NanoLuc®-RIPK2 HEK293 Cell Line	CS1810C01	2 vials
MAPK1 NanoBRET™ TE HEK293 Cell Line	CS1810C07	2 vials
IRAK3 NanoBRET™ TE HEK293 Cell Line	CS1810C41	2 vials

NanoBRET™ TE Thaw-and-Use NanoLuc® Cells

	Cat. #	Quantity
NanoLuc®-CRBN TE HEK293 Thaw-and-Use	CS1810E20 CS1810E21	1 vials 5 vials
VHL-NanoLuc® TE HEK293 Thaw-and-Use	CS1810E23 CS1810E24	1 vials 5 vials

Additional Thaw-and-Use cells are available for NanoLuc®-kinase fusions. Please inquire.

NanoBRET™ TE Detection Reagents

	Cat. #	Quantity
Intracellular TE Nano-Glo® Substrate/Inhibitor • NanoBRET™ Nano-Glo® Substrate • Extracellular NanoLuc® Inhibitor	N2162 N2160 N2161	100 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
Intracellular TE Nano-Glo® Vivazine™/Inhibitor • Nano-Glo® Vivazine™ Substrate • Extracellular NanoLuc® Inhibitor	N2200 N2201	1,000 assays (96-well) 10,000 assays (96-well)

Product Order Information

NanoBRET™ Protein:Protein Interaction (PPI) (page 9 – 11)

NanoBRET™ Ternary Complex Assays

	Cat. #	Quantity
NanoBRET™ CRBN Ternary Complex Starter Kit	ND2720	200 assays (96-well)
• HaloTag®-CRBN Fusion Vector (N2691) • pNLF1-N [CMV/Hygro] Vector (N1351) • pNLF1-C [CMV/Hygro] Vector (N1361) • HaloTag® Control Vector (G6591) (Negative Control) • NanoLuc®-BRD4 FL Fusion Vector (N1691) (Postive Control) • NanoBRET™ Nano-Glo® Detection System (N1661)		
NanoBRET™ VHL Ternary Complex Starter Kit	ND2700	200 assays (96-well)
• HaloTag®-VHL Fusion Vector (N2691) • pNLF1-N [CMV/Hygro] Vector (N1351) • pNLF1-C [CMV/Hygro] Vector (N1361) • HaloTag® Control Vector (G6591) (Negative Control) • NanoLuc®-BRD4 FL Fusion Vector (N1691) (Postive Control) • NanoBRET™ Nano-Glo® Detection System (N1661)		

In case the target is a kinase, BET-family or HDAC protein, refer to our huge selection of pre-cloned NanoLuc® fusion vectors.

NanoBRET™ Ubiquitination Assay

	Cat. #	Quantity
NanoBRET™ Ubiquitination Starter Kit	ND2690	200 assays (96-well)
• HaloTag®-Ubiquitin Fusion Vector (N2721) • pNLF1-N [CMV/Hygro] Vector (N1351) • pNLF1-C [CMV/Hygro] Vector (N1361) • HaloTag® Control Vector (G6591) (Negative Control) • NanoLuc®-BRD4 FL Fusion Vector (N1691) (Postive Control) • NanoBRET™ Nano-Glo® Detection System (N1661)		

Ready-to-use assays for distinct targets (e.g. β-catenin, c-Myc) available, please inquire.

Product Order Information

NanoBRET™ Proteasomal Recruitment Assay

	Cat. #	Quantity
NanoBRET™ Proteasomal Recruitment Starter Kit • HaloTag®-PSMD3 Fusion Vector (N2701) • pNLF1-N [CMV/Hygro] Vector (N1351) • pNLF1-C [CMV/Hygro] Vector (N1361) • HaloTag® Control Vector (G6591) (Negative Control) • NanoLuc®-BRD4 FL Fusion Vector (N1691) (Postive Control) • NanoBRET™ Nano-Glo® Detection System (N1661)	ND2730	200 assays (96-well)

Ready-to-use assays for distinct targets (e.g. β-catenin, c-Myc) available, please inquire.

NanoBRET™ HaloTag® Fusion Vectors

	Cat. #	Quantity
E3 Ligases		
HaloTag®-CRBN Fusion Vector	N2691	20 µg
HaloTag®-VHL Fusion Vector	N2731	20 µg

Different E3 Ligase?

Collection of HaloTag-E3 Ligase Fusions (~320)

www.promega.com/FindMyGene

Other		
HaloTag®-Ubiquitin Fusion Vector	N2721	20 µg
HaloTag®-PSMD3 Fusion Vector	N2701	20 µg

NanoBRET™ Control Vectors

	Cat. #	Quantity
NanoLuc®-BRD4 FL Fusion Vector	N1691	20 µg
HaloTag® Control Vector	G6591	20 µg

Product Order Information

NanoBRET™ PPI Stable HaloTag® Cell Lines

	Cat. #	Quantity
HaloTag®-CRBN HEK293 Cell Line	CS3005A01	2 vials
HaloTag®-VHL HEK293 Cell Line	CS2016A02	2 vials

Empty Vectors for NanoLuc® Target Protein Fusions

	Cat. #	Quantity
MCS Vectors		
pNLF1-N [CMV/Hygro] Vector	N1351	20 µg
pNLF1-C [CMV/Hygro] Vector	N1361	20 µg
Flexi® Vectors		
pFN31A *Nluc* CMV-Hygro Flexi® Vector	N1311	20 µg
pFC32A *Nluc* CMV-Hygro Flexi® Vector	N1331	20 µg
pFN31K *Nluc* CMV-neo Flexi® Vector	N1321	20 µg
pFC32K *Nluc* CMV-neo Flexi® Vector	N1341	20 µg

NanoBRET™ PPI Detection Reagents

	Cat. #	Quantity
NanoBRET™ Nano-Glo® Detection System (< 2 h) • NanoBRET™ Nano-Glo® Substrate • HaloTag® NanoBRET™ 618 Ligand	N1661 N1662 N1663	200 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well)
NanoBRET™ Nano-Glo® Kinetic Detection System (> 2 h) • Nano-Glo® Vivazine™ Substrate • HaloTag® NanoBRET™ 618 Ligand	N2583 N2584 N2585	200 assays (96-well) 1,000 assays (96-well) 10,000 assays (96-well))

Need help with the design of your CRISPR/HiBiT tagging experiment?
Want to get a free recommendation for crRNA and donor DNA sequences?

please contact our technical service

www.promega.com/support/tech-support

For Research Use Only. Not for Use in Diagnostic Procedures.

Helpful Resources

Targeted Protein Degradation

- Caine, E.A. *et al.* (2020) *Targeted Protein Degradation Phenotypic Studies Using HaloTag CRISPR/Cas9 Endogenous Tagging Coupled with HaloPROTAC3.* Curr Protoc Pharmacol 91(1):e81
- Riching, K.M. *et al.* (2020) *High-Throughput Cellular Profiling of Targeted Protein Degradation Compounds using HiBiT CRISPR Cell Lines.* J. Vis. Exp. (165), e61787
- Zhang, Y. *et al.* (2019) *Targeted protein degradation mechanisms.* Drug Discov Today Technol. 31:53-60
- Zoppi, V. *et al.* (2019) *Iterative Design and Optimization of Initially Inactive Proteolysis Targeting Chimeras (PROTACs) Identify VZ185 as a Potent, Fast, and Selective von Hippel-Lindau (VHL) Based Dual Degrader Probe of BRD9 and BRD7.* J Med Chem. 62(2):699-726
- Daniels, DL. *et al.* (2019) *Monitoring and deciphering protein degradation pathways inside cells.* Drug Discov Today Technol. 31:61-68
- Riching, K.M. *et al.* (2018) *Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action.* ACS Chem Biol. 13(9):2758-2770
- Buckley, DL. *et al.* (2015) *HaloPROTACS: use of small molecule PROTACs to induce degradation of HaloTag fusion proteins.* ACS Chem Biol. 10(8):1831-7

NanoBRET™ TE & PPI

- Guo, WH *et al.* (2020) *Enhancing intracellular accumulation and target engagement of PROTACs with reversible covalnet chemistry.* Nat Commun. 11(1):4268
- Wells, C *et al.* (2020) *Quantifying CDK inhibitor selectivity in live cells.* Nature Communications 11(1):2743
- Dale, NC. *et al.* (2018) *NanoBRET: The Bright Future of Proximity-Based Assays.* Front Bioeng Biotechnol. 7:56
- Vasta, JD. *et al.* (2018) *Wide-Spectrum Kinase Profiling in Live Cells for Assessing the Effect of cellular ATP on Target Engagement.* Cell Chem Biol. 25:1-9
- Robers, MB. *et al.* (2015) *Target engagement and drug residence time can be observed in living cells with BRET.* Nat Commun. 6:10091
- Machleidt, T. *et al.* (2015) *NanoBRET – A Novel BRET Platform for the Analysis of Protein:Protein Interactions.* ACS Chem Biol. 10(8):1797-804

HiBiT Protein Tagging System

- Schwinn, MK. *et al.* (2020) *A simple and scalable strategy for analysis of endogenous protein dynamics.* Sci Rep. 10(1):8953
- Schwinn, M.K. *et al.* (2017) *CRISPR-Mediated Tagging of Endogenous Proteins with a Luminescent Peptide.* ACS Chem Biol. 13(2):467-474

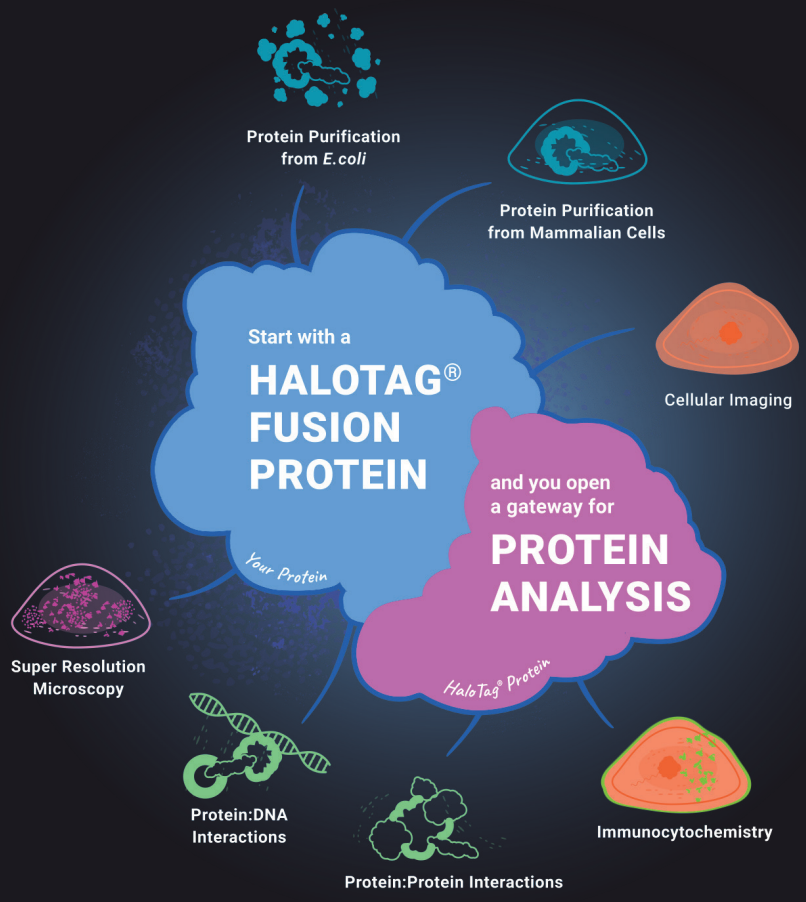
HaloTag® Technology

- Los, GV. et al. (2008) *HaloTag: a novel protein labeling technology for cell imaging and protein analysis.* ACS Chem Biol. 3(6):373-82

Promega

HaloTag® Technology

A Powerful Tool for Protein Labeling and Analysis



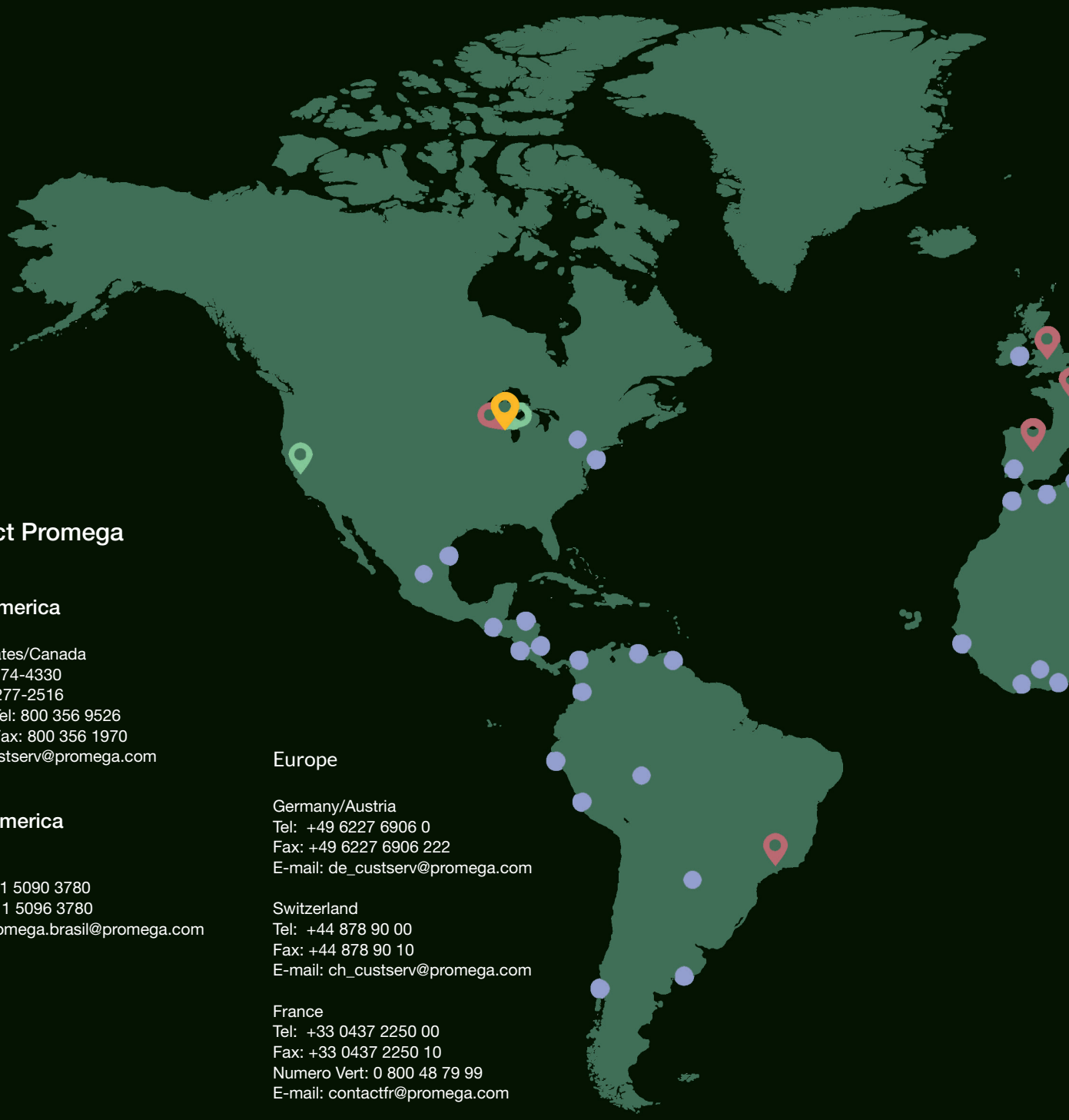
Three Small Steps. It's that easy!

1. Add HaloTag® fusion tag to your protein
2. Pair with one of many HaloTag® Ligands
3. Ready to access numerous application

Start right away by going to **www.promega.com/FindMyGene** and choose from > 9.000 validated HaloTag®-human ORF clones

Find out more and visit
www.promega.com/halotag-technology

Worldwide Promega Contact Details



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

CRISPR/Cas9 Knock-In Clones & Pools

N- or C-terminal fusion with reporter tag

Multiple reporter tags to suit your needs

Choose cell background of interest

Screen our growing selection of target genes

Cells are available as edited pools (P) and/or stable cell clones (C)

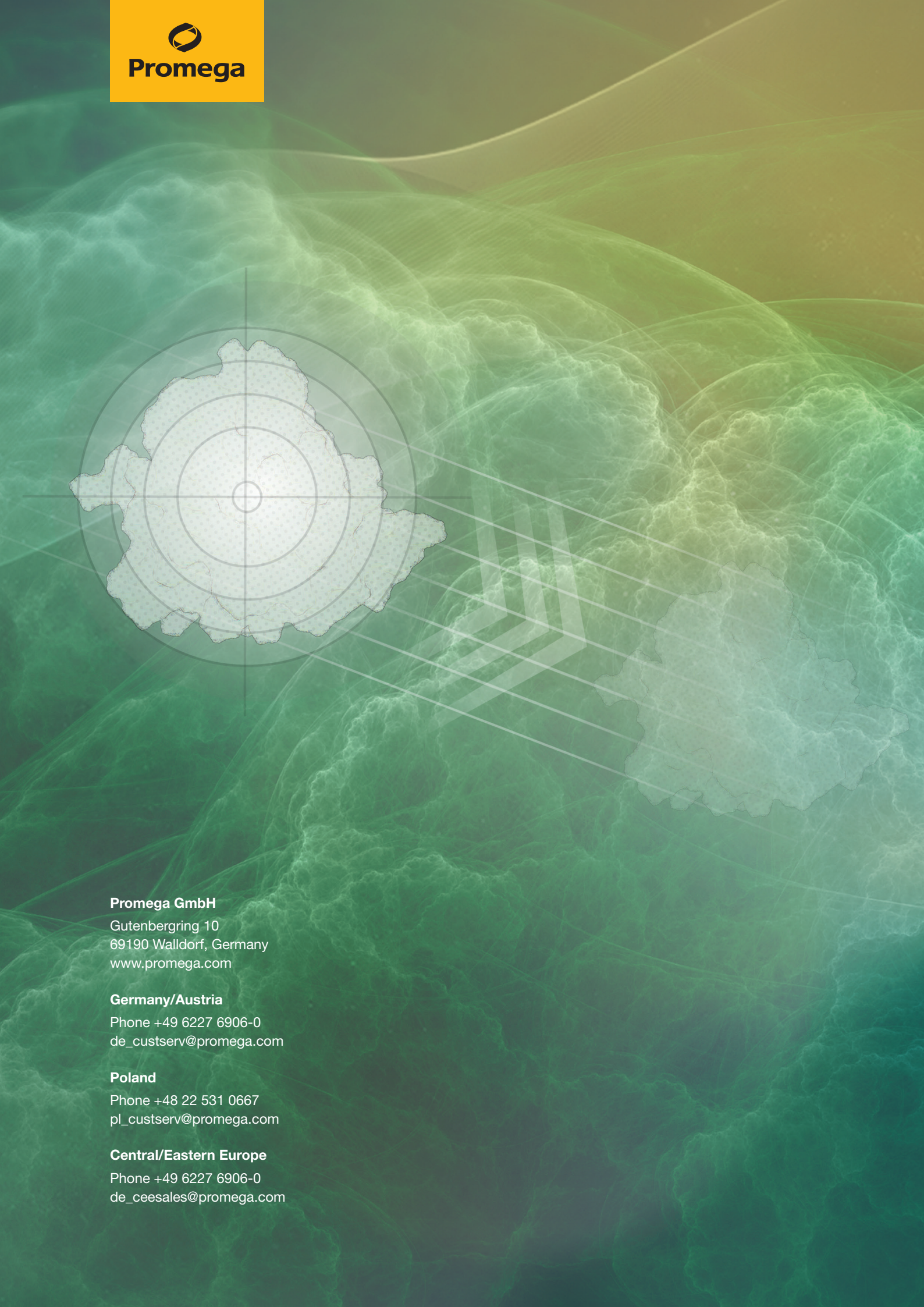
Target	Tag	Term	Background	Clone
BRAFV600E	HiBiT	N	A375	C
BRD4	HiBiT	N	A-549	C
BRD4	HiBiT	N	HEK293	C
BRD4	HiBiT	N	HEK293 LgBiT	C
BRD4	HiBiT-HaloTag®	N	HEK293	P
BRD4	HiBiT-HaloTag®	N	HEK293 LgBiT	C/P
BRD4	NanoLuc®	N	HEK293	C

Promega continues to expand the collection of HiBiT, NanoLuc®, and HaloTag® CRISPR knock-in cell pools and clones. To see the most recent list of available content please visit:

www.promega.com/CRISPR_HiBiT

Questions about a cell line, or don't see your target or cell background of interest?

Please speak to your local Promega representative or reach out to our Elite Access group: EliteAccess@promega.com



Promega GmbH
Gutenbergring 10
69190 Walldorf, Germany
www.promega.com

Germany/Austria
Phone +49 6227 6906-0
de_custserv@promega.com

Poland
Phone +48 22 531 0667
pl_custserv@promega.com

Central/Eastern Europe
Phone +49 6227 6906-0
de_ceesales@promega.com