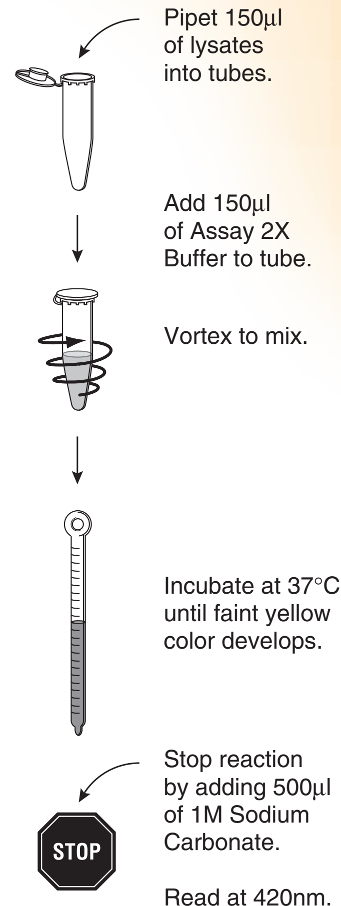
β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer

INSTRUCTIONS FOR USE OF PRODUCT E2000.

Quick PROTOCOL

Standard Protocol
(96-well plate protocol on reverse side)

1. Prepare cell lysates (see Section 3 of the Technical Bulletin).
2. Thaw system components and mix well. Place 2X Assay Buffer on ice.
3. It may be necessary to dilute cell lysates in 1X Reporter Lysis Buffer (RLB). A 2:1 dilution of lysate to 1X RLB (100µl of lysate plus 50µl of 1X RLB) is a good starting dilution, but up to 150µl of cell lysate can be used per reaction.
4. Prepare a negative control (lysate from nontransfected cells) in 1X RLB, using the same dilution as in Step 3.
5. Add 150µl of Assay 2X Buffer to each tube. Mix by vortexing briefly.
6. Incubate reactions at 37°C for 30 minutes or until a faint yellow color has developed.
7. Stop reactions by adding 500µl of 1M Sodium Carbonate. Vortex briefly to mix.
8. Read the absorbance at 420nm.



For additional protocol information, see Technical Bulletin #TB097, available online at: **www.promega.com/tbs**

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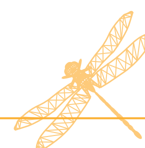

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Printed in USA. Revised 3/09.
Part #9FB051

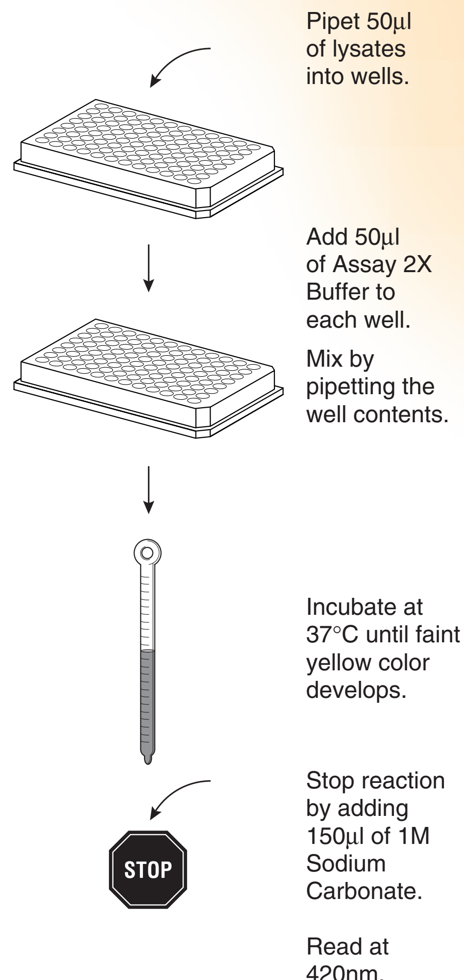
β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer

INSTRUCTIONS FOR USE OF PRODUCT E2000.

Quick PROTOCOL

96-Well Plate Assay

1. Prepare cell lysates (see Section 3 of the Technical Bulletin).
2. Thaw system components and mix well. Place 2X Assay Buffer on ice.
3. It may be necessary to dilute cell lysates in 1X Reporter Lysis Buffer (RLB). Mix 30µl of lysate with 20µl of 1X RLB, as a starting dilution (as much as 50µl of cell lysate can be used per reaction). Pipet 50µl of cell lysates into wells of a 96-well plate.
4. Prepare a negative control, using nontransfected cells, in 1X RLB at the same dilution as used in Step 3.
5. Add 50µl of Assay 2X Buffer to each well.
6. Mix samples by pipetting the contents of each well. Cover the plate.
7. Incubate the plate at 37°C for 30 minutes or until a faint yellow color has developed. Due to the small sample volumes in the plate, do not incubate reactions overnight.
8. Stop reactions by adding 150µl of 1M Sodium Carbonate. Mix by pipetting the contents of each well gently to avoid bubbles.
9. Read the absorbance at 420nm.



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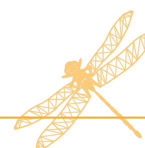

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