The Beta-Glo® Assay System provides a sensitive luminescent reagent for detecting and quantifying β-galactosidase activity in a homogeneous assay format. It is brighter and more sensitive than any other currently available luminescence-based β-galactosidase assay. After reaching peak activity, the reaction exhibits steady-state kinetics for several hours, making it suitable for high-throughput screening. The assay is linear over a wide range of cell number, spanning at least four orders of magnitude of enzyme activity. This homogeneous format is amenable to any multiwell plate format and can be used with mammalian, yeast or bacterial cells.

Chemistry and Assay Procedure

The Beta-Glo® Assay can be used to measure β-galactosidase activity in a variety of biological applications, including complementation studies involving protein:protein interaction and yeast two-hybrid screening. The Beta-Glo® Assay System(a,b) is a homogeneous bioluminescent assay that couples β-galactosidase activity to a luciferase reaction (Figure 1). β-galactosidase catalyzes a reaction in which the substrate (D-luciferin-α-β-galactopyranoside) is cleaved to release luciferin. This luciferin serves as a substrate for luciferase that is present in the reagent. As a result of the luciferase activity, oxyluciferin is formed, and light is emitted. The luminescence observed is proportional to the amount of β-galactosidase present. The reagent has been carefully formulated so that the assay is a single-step procedure that involves adding an equal volume of reagent to a sample that contains the enzyme either in solution or present in cells grown in medium and serum. For the latter purpose, the reagent also contains a detergent that lyses cells to release the β-galactosidase present.

Compatibility with Mammalian and Yeast Cells

The Beta-Glo® Assay was developed for use with mammalian cells. We used a cell line (ψ2BAGα) that was derived from NIH3T3 cells and stably transfected with bacterial β-galactosidase. We show that the luminescence produced is linear from 3 cells to 30,000 cells per well in a 96-well plate (Figure 2). The luminescence is stable, and there is virtually no loss in activity from 30 minutes to 4 hours after adding the reagent (2). The stable kinetics allows processing of multiple plates at a time.

Additionally, the reagent was tested for its ability to be used with yeast cells; it was shown to be an effective reagent for measuring β-galactosidase activity in the yeast two-hybrid system, even in a highly miniaturized format. Figure 3A shows that the reagent can lyse yeast cells and is also brighter than another commercially available reagent specifically designed for use with yeast cells; the Beta-Glo® Assay also appears to have greater sensitivity. This is particularly important for detection of weaker interactions where a less sensitive reagent might be unable to detect low signals (Figure 3B). The signal from a yeast two-hybrid assay was shown to be stable between the recommended time point after Beta-Glo® Reagent addition (25 minutes) to over one hour (Figure 4).

Figure 1. Summary of the coupled reactions in the Beta-Glo® Assay System. β-galactosidase activity from lysed cells or in solution catalyzes the conversion of D-luciferin-α-β-galactopyranoside to D-luciferin, which is in turn a substrate for luciferase.
The reagent has been carefully formulated so that the assay is a single-step procedure.
Compatibility with Bacterial Cells and Lysates

Preliminary testing with JM109 bacterial cells containing the pGEM®3Zf(+) Vector© indicated that the Beta-Glo® Assay can lyse bacterial cells in Luria Broth (LB) growth medium. The assay for β-galactosidase activity was carried out at varying densities of bacterial cells. The emitted light was proportional to the number of cells present (based on OD 600; Figure 5). Neither the addition of lysozyme nor a freeze-thaw step increased the β-galactosidase signal with these bacterial cells (data not shown).

Additionally, the reagent was also tested for compatibility with various buffers. Figure 6 shows that the assay works well for β-galactosidase activity in buffers used for sample preparation or in media used for growing cells. The Beta-Glo® Assay was also tested against several lysis buffers (Promega reagents) that are used to prepare cell lysates for reporter assays. The most compatible buffers appear to be H/P

References


Protocol


Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat.#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Glo® Assay System(b)</td>
<td>10ml</td>
<td>E4720</td>
</tr>
<tr>
<td></td>
<td>100ml</td>
<td>E4740</td>
</tr>
<tr>
<td></td>
<td>10 × 100ml</td>
<td>E4780</td>
</tr>
</tbody>
</table>

(a)Certain applications of this product may require licenses from others.
(b)The method of recombinant expression of Coleoptera luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
(c)U.S. Pat. No. 4,766,072.

Beta-Glo is a trademark of Promega Corporation. pGEM is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office.
CircleGrow is a registered trademark of QBiogene, Inc. MLX is a registered trademark of Dynex Technologies, Inc.
Prionex is a registered trademark of Pentapharm, Ltd. ViewLux is a trademark of Perkin Elmer Life Sciences, Inc.