Amplite™ Fluorimetric beta-Galactosidase Assay Kit
Green Fluorescence

Ordering Information:
Product Number: #12061 (500 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in freezer (Component A)/refrigerator and avoid light

Introduction

E. coli beta-galactosidase is a 464 kD tetramer. Each unit of beta-galactosidase consists of five domains, the third of which is an active site. It is an essential enzyme in the human body. Deficiencies in the protein can result in galactosialidosis or Morquio B syndrome. In E. coli, beta-galactosidase is produced by activation of the LacZ operon. Detection of LacZ expression has become routine to the point of detection of as few as 5 copies of beta-galactosidase per cell.

This kit uses a fluorogenic galactosidase substrate that can sensitively distinguish LacZ+ vs. LacZ- cells. It can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells. The galactosidase-cleaved product has Ex/Em = 490/520 nm that can detected with most of fluorescence instruments equipped with a FITC filter set.

Kit Key Features

Sensitive:	Can detect galactosidase activities in a few cells
Continuous:	Suitable for manual or automated operations with no mixing or separations.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Component	Amount
Component A: Fluorescein di-beta-D-galactopyranoside (FDG)	1 vial
Component B: Reaction buffer	1 bottle (100 ml)
Component C: Stop buffer	1 vial (25 mL)
Component D: 5X lysis buffer	1 vial (10 mL)
Component E: DMSO	1 vial (500 µL)
Component F: β-mercaptoethanol	1 vial (500 µL)

Materials Required (but not provided)

- 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom.
- Fluorescence microplate reader: Capable of detecting Ex=490nm/Em=520-530nm.
- β-galactosidase (E. Coli)

Assay Protocol (for 1 plate)

Brief Summary

Stable or transient transfected cells with LacZ gene→ Prepare cells (samples) with test compounds → Lyze the cells → Transfer the lysate to a microtiter plate → Add FDG working solution →Incubate at room temperature or 37°C for 5 min to hours → Add stopping solution → Read Fluorescence at Exc=490nm/Emi=525nm

1. Prepare FDG Working Solution for 1 plate

1.1 Thaw all the components to room temperature before use.
1.2 Make FDG stock solution by adding 250 ul Component E (DMSO) into Component A (FDG).
Note: 25 ul of FDG is enough for 1 plate, aliquot and stored un-used FDG at ≤ -20°C, avoiding light and repeated freeze-thaw cycles.
1.3 Make 0.3 % β-mercaptoethanol assay buffer by adding 30 µL of Component F (β-mercaptoethanol,) to 10 mL of Component B (reaction buffer), and mix well.
Note: additional buffer is needed for preparing enzyme dilution buffer, which is used when generating a standard curve
1.4 Make FDG working solution by adding 25 µL of FDG stock solution (from step 1.2) into 10 mL 0.3 % β-mercaptoethanol assay buffer (from step 1.3)
Note1: DO not expose FDG solutions to room temperature for extended periods of time as spontaneous hydrolysis will occur.
Note2: un-used FDG solutions can be aliquoted and stored at ≤ -20°C for more than one month, avoiding light and repeated freeze-thaw cycles.

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2. Preparation of 1X Lysis buffer

2.1 Make 1X lysis buffer by diluting the Component D (5X Lyses buffer) into 80 mL H2O. Unused 1X lysis buffer can be stored at 4°C for future use. Add 0.1% Component F (β-mercaptoethanol) before use.
Note: Always add 0.1% β-mercaptoethanol to 1X lysis buffer before lysising the cells.

3. Preparation cell extracts from mammalian cells

3.1 Treat cells with test compounds for a desired period of time.
3.2 Wash the cells 2 times with 1X PBS. Do not dislodge the cells.
3.3 For adherent cells, add 1X lysis buffer (from step 2.1) to the culture plates. Recommended volumes for various plates are listed in the following table.

Type of culture Plate	Volume of 1X Lysis buffer (uL/well)
96-well plate	50
24-well plate	250
12-well plate	500
6-well plate	1000
60 mm plate	2000
100 mm plate	4000

For non-adherent cells, pellet the cells into centrifuge tube, then add 50- 2000 ul (depends on the size of the cell pellet) of 1X lysis buffer to the tube.

3.4 Incubate the cells with cell lysis buffer for 10-15 min at room temperature, gently swirl the plates or tubes several times to ensure complete lysis.

3.5 Proceed to the FDG assay or freeze the sample at -80^{o}C till use.
Note1: A good lysis can also be obtained by a quick freeze/thaw cycle (freeze 1-2 hours at -20^{o}C to -80^{o}C and thaw at room temperature.
Note 2: Alternatively, centrifuge the cell lysis for 2-3 minutes to pellet the insoluble material, then assay the supernatant.

4. Run 96-well plate β-Galactosidase assay

4.1 Thaw the tube or plate of lysed cells at room temperature if needed. Perform the assay directly on the 96-well plate if the cells were plated with a 96-well plate.

4.2 Add 50 ul of cell extracts (from step 3.4) to 96 wells. Save some control wells for the standard curve if a standard curve is desired.

4.3 Optional (if a standard curve is desired): Prepare a serial dilution of β-galactosidase (E. Coli) standards with 0.3 % β-mercaptoethanol assay buffer (from step 1.4). Transfer 50 µL aliquot of each point on the standard curve to the control wells of the plate. The highest recommended amount of β-galactosidase is 200 milliunits (200,000-400,000 pg). 2X serial dilution of standard curve consisting of 8 points is recommended. A dilution procedure example is shown in the following table.

β-gal Standard (milliunits)	Assay Buffer Volume	β-gal Standard volume
200	990 ul	10 ul of 20 units β-gal standard stock
100	200 ul	200 ul of 200 mu ß-gal standard
50	200 ul	200 ul of 100 mu ß-gal standard
25	200 ul	200 ul of 50 mu ß-gal standard
12.5	200 ul	200 ul of 25 mu ß-gal standard
6.25	200 ul	200 ul of 12.5 mu ß-gal standard
3.125	200 ul	200 ul of 6.25 mu ß-gal standard
1.562	200 ul	200 ul of 3.125 mu ß-gal standard

Note 1: Adjust the standard curve to suit the specific experimental conditions, such as cell type, number, transfection effeciency, size of the culture plates.
Note 2: The dilutions for the standard curve must be freshly prepared for the assay

4.4 Add 50 µL of each sample/well.
Note1: If necessary, dilute the lysate in 1X Lysis Buffer when transfection efficiency is very high. Or reduce the volume of lysis buffer used when transfection efficiency is low. If the transfection is performed with a 96-well plate, or a stable cell line was seed into a 96-well plate, perform the assay directly on the plate.
Note 2: For endogenous ß-galactosidase activity control, add 50 µL of cell lysate from non-transfected cells. For blank control, add 50 µL of 1X lysis buffer

4.5 Add 50µL of of FDG working solution (from step 1.4) to each plate. Incubate the plate at room temperature or 37°C (from approximately 10 min. to 4 h depending on the cell type).
4.6 Add 50 µL of Component C (stop buffer) to each well. The stop buffer causes an increase in the fluorescence of the product, in addition to terminate the reaction.
4.7 Measure the fluorescence of the solution in each well using a fluorescence microplate reader with excitation at 490nm and emission at 520nm.
4.8 Quantify ß-galactosidase expression based on a linear standard curve.

Data Analysis

The fluorescence in blank wells with the assay buffer and FDG working solution is used as a control, and is subtracted from the values for the cell (or sample) wells. The background fluorescence of the blank wells can be varied depending upon the sources of the microtiter plates. The typical data are included in Figure1 (β-galactosidase (E. Coli) titration curve).

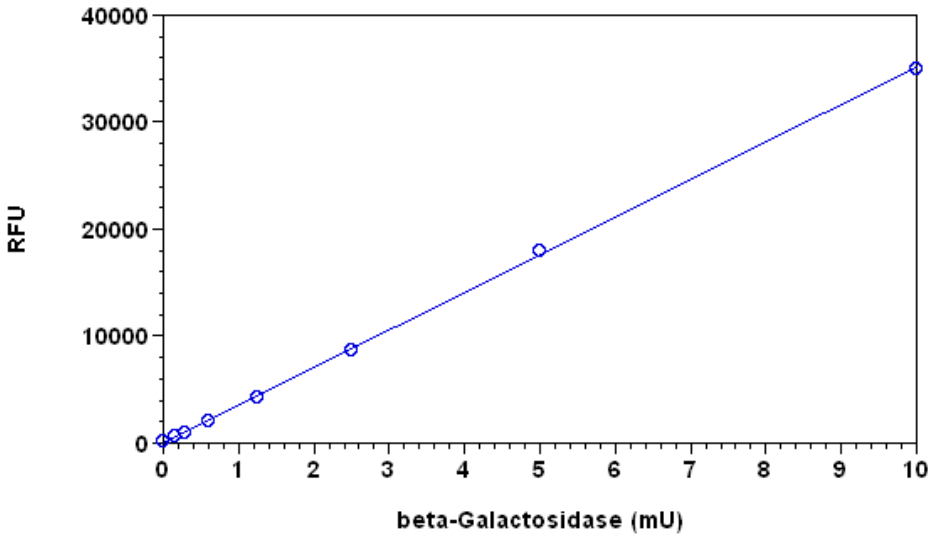

Figure 1. β-galactosidase dose response was measured with Amplite™ Fluorimetric beta-Galactosidase Assay Kit on Costar 96-well black plate using a NOVOstar plate reader (BMG LabTech). As low as 0.15 mU β-galactosidase can be detected with 30 minutes incubation time.

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