Amplite™ Fluorimetric Beta-Galactosidase Assay Kit *Green Fluorescence*

Catalog number: 12601
Unit size: 500 Tests

Component	Storage	Amount
Component A: Fluorescein di-β-D-Galactopyranoside (FDG)	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial
Component B: Reaction Buffer	Freeze (<-15 °C), Avoid Light	1 bottle (50 mL)
Component C: Stop Buffer	Freeze (<-15 °C), Avoid Light	1 vial (25 mL)
Component D: Lysis Buffer	Freeze (<-15 °C), Avoid Light	1 vial (25 mL)
Component E: DMSO	Freeze (<-15 °C), Avoid Light	1 vial (500 µL)
Component F: β-Mercaptoethanol	Freeze (<-15 °C), Avoid Light	1 vial (500 µL)

OVERVIEW

E. coli beta-galactosidase is a 464 kD tetramer. Each unit of beta-galactosidase consists of five domains, the third of which is the active site. It is an essential enzyme in cells. Deficiencies in this enzyme can result in galactosialidosis or Morquio B syndrome. In E. coli, beta-galactosidase is produced by the activation of LacZ operon. Detection of LacZ expression has become routine to the point of detection of as few as 5 copies of ?-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 be detected with most of fluorescence instruments equipped with a FITC filter set.

AT A GLANCE

Protocol summary

1. Prepare stable or transient transfected cells with LacZ gene
2. Incubate cells (samples) with test compounds
3. Lyse the cells
4. Transfer the lysate to a microtiter plate
5. Add FDG working solution
6. Incubate at room temperature or 37°C for at least 5 minutes depending on cell type
7. Add stopping solution
8. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Thaw all the kit components to room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. FDG stock solution (1X):
Add 125 µL of DMSO (Component E) into the vial of FDG (Component A) to make 1X FDG stock solution.

Note 25 µL of FDG is enough for 1 plate. Keep from light.

PREPARATION OF STANDARD SOLUTION

β-Galactosidase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/12601

Optional (if a standard curve is desired): Prepare a serial dilution of β-galactosidase (E. Coli) standards with 0.3% β- mercaptoethanol assay buffer. 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 mU/mL (200 - 400 ng). 2X serial dilution of standard curve consisting of 8 points is recommended.

Note Adjust the standard curve to suit the specific experimental conditions, such as cell type, number, transfection effeciency, and size of the culture plates. The dilutions for the standard curve must be prepared freshly each time the assay is performed.

PREPARATION OF WORKING SOLUTION

1. 0.3 % β-mercaptoethanol assay buffer:
Add 30 µL of β-mercaptoethanol (Component F) to 10 mL of Reaction Buffer (Component B), and mix well.
Note: Additional buffer is needed for preparing enzyme dilution buffer, which is used to generate a standard curve.

2. FDG working solution:
Add 25 µL of 1X FDG stock solution into 5 mL of 0.3 % β-mercaptoethanol assay buffer.

Note DO NOT keep FDG solutions at room temperature for an extended period of time as spontaneous hydrolysis will occur.

3. Lysis buffer working solution:
Add 5 µL of β-mercaptoethanol (Component F) to 5 mL of Lysis Buffer (Component D) before use.

Note Always add 0.1% β-mercaptoethanol into lysis buffer before lysing the cells

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Recommended Lysis Buffer working solution volumes for cell culture plates.

Type of culture plates	Lysis Buffer working solutions (µL/well)

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96-well plate	50
24-well plate	250
12-well plate	500
6-well plate	1000
60 mm plate	2000
100 mm plate	4000

Prepare cell extracts from mammalian cells

1. Treat cells containing LacZ gene with test compounds for a desired period of time.

2. Wash the cells twice with 1X PBS. Do not dislodge the cells.

3. Lyse cells accordingly with Lysis Buffer working solution.

   For adherent cells; Add Lysis Buffer working solution to the culture plates. See table 1 for recommended volumes.

   For non-adherent cells: Pellet the cells into centrifuge tube, and add 50 - 2000 µL (depending on the size of the cell pellet) of Lysis Buffer working solution to the tube.

4. Incubate cells from previous step at room temperature for 10 - 15 minutes, and gently swirl the plates or tubes several times to ensure complete lysis.

5. Proceed to the FDG assay or freeze the sample at -80 °C until use.

   Note A good lysis can also be obtained by a quick freeze-and-thaw cycle (freeze 1 - 2 hours at -20°C to -80°C and thaw at room temperature). Alternatively, centrifuge the cell lysis for 2 - 3 minutes to pellet the insoluble material, and then assay the supernatant.

Run ß-galactosidase assay

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 seeded in a 96-well plate.

2. Add 50 µL of cell extracts into each well of the 96-well plate. Save some control wells for the standard curve (50 uL/well) if a standard curve is desired.

   Note If necessary, dilute the lysate in Lysis Buffer working solution when transfection efficiency is very high or reduce the volume of lysis buffer when transfection efficiency is low. If the transfection is performed in a 96-well plate, or a stable cell line was seeded into a 96-well plate, perform the assay directly on the plate. For endogenous β-galactosidase activity control, add 50 µL of cell lysate from non-transfected cells. For blank control, add 50 µL of 1X lysis buffer.

3. Add 50 µL of FDG working solution to each well. Incubate the plate at room temperature or 37°C for approximately 5 min to 4 hr depending on the cell type.

4. Add 50 µL of Stop Buffer (Component C) to each well. The stop buffer causes an increase in the fluorescence intensity of the product, in addition to terminate the reaction.

5. Measure the fluorescence intensity of the solution in each well with a fluorescence microplate reader at Ex/Em = 490/525 nm.

6. Quantify ß-galactosidase expression based on a linear standard curve.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate β-Galactosidase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator/

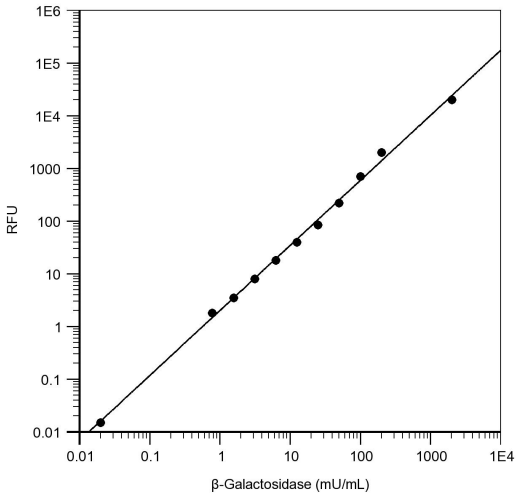

Figure 1. β-galactosidase dose response was measured with Amplite™ Fluorimetric beta-Galactosidase Assay Kit in a Costar solid black 96-well plate using Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER