Amplite™ Fluorimetric β-Galactosidase Assay Kit
Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 12601 (500 assays)	Different storage conditions required	Fluorescence microplate readers

Introduction

E. coli β-galactosidase is a 464 kD tetramer. Each unit of β-galactosidase consists of five domains, the third of which is the active site. It is an essential enzyme in cells. Deficiencies of this enzyme can result in galactosialidosis or Morquio B syndrome. In E. coli, β-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 β-galactosidase per cell.

This kit uses the fluorogenic fluorescein digalactoside (FDG) galactosidase substrate that can sensitively distinguish LacZ+ from LacZ- cells. The non-fluorescent substate generates the strongly fluorescent fluorescein upon reaction with galactosidase. It can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells. FDG used in the kit is not fluorescent. The galactosidase induced cleavage of FDG gives fluorescein that has the spectra of Ex/Em = 490/515 nm, which can be detected with most fluorescence instruments equipped with a FITC filter set. The kit comes with all the essential components with an optimized assay protocol. It can be used with a fluorescence microplate reader, a fluorescence microscope, or a flow cytometer. It might also be used for screening galactosidase inhibitors or inducers.

Kit Key Features

Sensitive: Detect galactosidase activities in a few cells.
Continuous: Suitable for both manual and automated operations without a mixing or separation step.
Convenient: Formulated to have minimal hands-on time.
Non-Radioactive: No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Fluorescein di-β-D-Galactopyranoside (FDG)	1 vial
Component B: Reaction Buffer	1 bottle (50 mL)
Component C: Stop Buffer	1 vial (25 mL)
Component D: Lysis Buffer	1 vial (25 mL)
Component E: DMSO	1 vial (500 µL)
Component F: β-Mercaptoethanol	1 vial (500 µL)

Note: Keep Component A in freezer and all the other components (B, C, D, E and F) at 4 ^{o}C for convenience.

Materials Required (but not provided)

- 96 or 384-well microplates: Tissue culture microplate with black wall and clear bottom.
- A fluorescence microplate reader: Capable of detecting Ex/Em = 490/525 nm.
- β-galactosidase (E. Coli)

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Assay Protocol for One 96-well Plate

Brief Summary

Prepare stable or transient transfected cells with LacZ gene → Incubate cells (samples) with test compounds → Lyse the cells → Transfer the lysate to a microtiter plate → Add FDG working solution →Incubate at room temperature or 37 °C for 5 minutes to hours → Add stopping solution → Monitor fluorescence intensity at Ex/Em = 490/525 nm

1. Prepare FDG working solution for 1 plate:

1.1 Thaw all the components at room temperature before use.

1.2 Make FDG stock solution: Add 125 µL of DMSO (Component E) into the vial of FDG (Component A).
Note: 25 µL of FDG is enough for 1 plate. Un-used FDG stock solution should be aliquoted and stored at ≤ -20 °C. Keep from light and avoid repeated freeze-and-thaw cycles.

1.3 Make 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.

1.4 Make FDG working solution: Add 25 µL of FDG stock solution (from Step 1.2) into 5 mL of 0.3 % β-mercaptoethanol assay buffer (from Step 1.3).
Note1: DO not keep FDG solutions at room temperature for an extended period 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. Keep from light and avoid repeated freeze-and-thaw cycles.

2. Prepare lysis buffer working solution:

Make lysis buffer working solution by adding 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.

3. Prepare cell extracts from mammalian cells:

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

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

3.3 For adherent cells: Add lysis buffer working solution (from Step 2) to the culture plates. Recommended volumes for various plates are listed in the following table.

Type of culture plate	Volume of lysis buffer working solution (µL/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, and add 50-2000 µL (depending on the size of the cell pellet) of 1X lysis buffer to the tube.

3.4 Incubate the cells with cell lysis buffer (from Step 3.3) at room temperature for 10-15 minutes, and gently swirl the plates or tubes several times to ensure complete lysis.

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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-and-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, and then assay the supernatant.

4. Run 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 seeded in a 96-well plate.

4.2 Add 50 µL of cell extracts (from Step 3.4) into each well of the 96-well plate. 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 1.3). 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 (200-400 ng). 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 (mU)	Assay Buffer Volume	β-gal Standard Volume
200	990 µL	10 µL of 20 units β-gal
100	200 µL	200 µL of 200 mU ß-gal
50	200 µL	200 µL of 100 mU ß-gal
25	200 µL	200 µL of 50 mU ß-gal
12.5	200 µL	200 µL of 25 mU ß-gal
6.25	200 µL	200 µL of 12.5 mU ß-gal
3.125	200 µL	200 µL of 6.25 mU ß-gal
1.562	200 µL	200 µL of 3.125 mU ß-gal

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

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 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.
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 50 µL of FDG working solution (from Step 1.4) to each well. Incubate the plate at room temperature or 37 ^{o}C for approximately 10 min to 4 hr depending on the cell type.

4.6 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.

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

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 varies depending upon the sources of the microtiter plates. A β-galactosidase (E. coli) titration curve is shown in Figure1.

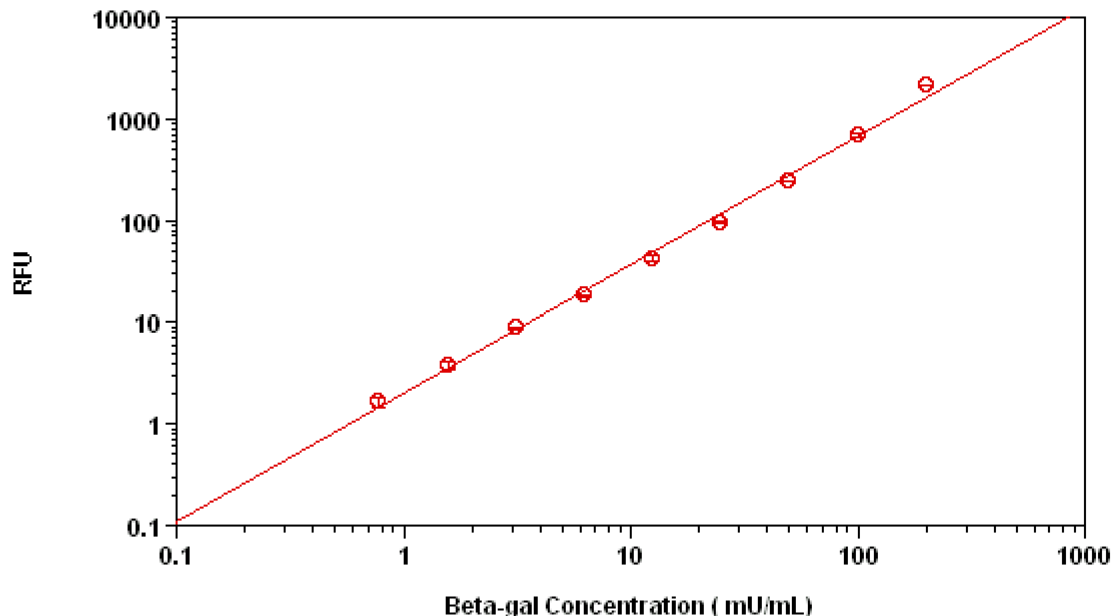

Figure 1. β-galactosidase dose response was measured with Amplite™ Fluorimetric beta-Galactosidase Assay Kit in a Costar black 96-well plate using Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU β-galactosidase can be detected with 30 minutes incubation.

References

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