Amplite™ Colorimetric Beta-Galactosidase Assay Kit

Catalog number: 12604
Unit size: 200 Tests

Component	Storage	Amount
Component A: Resorufin β-D-Galactoside	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Reaction Buffer	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (20 mL)
Component C: Stop Buffer	Freeze (<-15 °C), Minimize light exposure	1 vial (10 mL)
Component D: Lysis Buffer	Freeze (<-15 °C), Minimize light exposure	1 vial (10 mL)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)
Component F: β-Mercaptoethanol	Freeze (<-15 °C), Minimize light exposure	1 vial (100 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 of 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 beta-galactosidase per cell. This kit uses a chromogenic galactosidase substrate that can sensitively distinguish LacZ+ from LacZ- cells. The light yellow substrate generates a strongly purple product 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. Amplite™ Colorimetric Beta-Galactosidase Assay Kit comes with all the essential components with an optimized assay protocol. It can be used for screening galactosidase inhibitors or inducers.

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. Measure the OD ratio at the wavelength of 580 nm to 460 nm (Ab_{580}/Ab_{460})

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	580/460 nm
Recommended plate:	Clear bottom

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. Resorufin β-D-Galactosidase stock solution (200X):
Add 50 µL of DMSO (Component E) into the vial of Resorfuin β-D-Galactosidase (Component A) to make 200X Resorufin β-D-Galactosidase stock solution.

Note 25 µL of this stock solution is enough for one 96-well 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/12604

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). 1:3 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. Resorufin βDG working solution:
Add 25 µL of Resorufin β-D-Galactosidase stock solution (200X) into 5 mL of 0.3 % β-mercaptoethanol assay buffer.

Note This working solution is enough for one 96-well plate.

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.

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Type of culture plates	Lysis Buffer working solutions (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

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 β-galactosidase 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 Lysis Buffer working solution.
3. Add 50 µL of Resorufin βDG working solution to each well. Incubate the plate at room temperature or 37°C for approximately 10 min to 4 hr depending on the cell type.
4. Add 50 µL of Stop Buffer (Component C) to each well.
5. Monitor the absorbance increase by measuring the OD ratio at the wavelength of 580 nm to 460 nm (Ab_{580}/Ab_{460}) using an absorbance microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs 580/Abs 460 (x1E-3)) 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

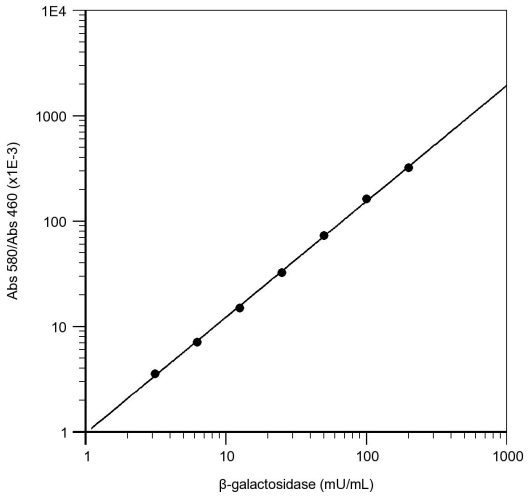

Figure 1. β -galactosidase dose response was measured with Amplite™ Colorimetric β-Galactosidase Assay Kit (Cat #: 12604) in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices). As low as 3 mU/mL β- galactosidase was detected with 30-60 minutes incubation. (Note: The absorbance background increases with time, thus it is important to subtract the absorbance of the blank wells for each data point.)

DISCLAIMER