Amplite™ Colorimetric β-Galactosidase Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 12604 (200 assays)	Refrigerated	Absorbance microplate reader

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.

Amplite™ Colorimetric β-Galactosidase Assay Kit offers a sensitive fluorescent assay to distinguish LacZ+ from LacZ- cells. The light yellow resorufin β-galactoside reacts with β-galactosidase to generate a purple color. The assay can be performed using an absorbance microplate reader by measuring the OD ratio at the wavelength of 580 nm to 460 nm. Our kit enables researchers to detect galactosidase conjugates in ELISA type assay systems, monitor LacZ gene expression in cells, or screen galactosidase inhibitors or inducers.

Kit Components

Component	Amount
Component A: Resorufin β-D-Galactoside	1 vial
Component B: Reaction Buffer	1 bottle (20 ml)
Component C: Stop Buffer	1 vial (10 mL)
Component D: Lysis Buffer	1 vial (10 mL)
Component E: DMSO	1 vial (100 µL)
Component F: β-Mercaptoethanol	1 vial (100 µL)

Notes: 1. To achieve the best results, it's strongly recommended to use the black plates.
2. Thaw one vial of each kit component at room temperature before starting the experiment.

Materials Required (but not provided)

- β-Galactosidase (*E. Coli*)

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 assay mix →Incubate at room temperature or 37 °C for 10 minutes to 4 hours → Add stop buffer→ Measure the OD ratio at the wavelength of 580 nm to 460 nm (Ab_{580}/Ab_{460})

1. Prepare assay mix for One 96-well plate:

1.1 Prepare resorufin β-D-galactoside substrate stock solution (200X): Add 50 µL of DMSO (**Component E**) into the vial of resorufin β-D-galactosidase (**Component A**) to make stock solution (200X).
Note: 25 µL of resorufin β-D-galactosidase stock solution (200X) is enough for one 96-well plate. Unused stock solution should be aliquoted and stored at ≤ -20 °C. Keep from light and avoid repeated freeze-and-thaw cycles.

1.2 Prepare 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 a serial dilution of β-galactosidase samples, which is used to generate a standard curve.

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1.3 Prepare assay mixture: Add 25 µL of resorufin β-D-galactosidase stock solution (from Step 1.1) into 5 mL of assay buffer (from Step 1.2) to make assay mixture.
Note 1: This β-Gal assay mixture is enough for one 96-well plate. It is not stable, use it promptly.
Note 2: One can divide unused β-Gal assay mixture into single use aliquots and stored at -20°C.

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.

3.5 Proceed to the β-galactosidase assay or freeze the sample at -80 °C till use.
Note1: 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).
Note 2: Alternatively, centrifuge the cell lysis for 2-3 minutes to pellet the insoluble material, and run tests on 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/well of β-galactosidase containing cell extracts (from Step 3.4) into a 96-well solid back plate.
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 β-galactosidase activity control, add 50 µL of cell lysate from non-transfected cells. For blank control, add 50 µL of lysis buffer.

4.3 *Optional* (if a standard curve is desired): Prepare β-galactosidase (E. Coli) standards by performing 1:3 serial dilutions in 0.3 % β-mercaptoethanol assay buffer (from Step 1.2) to get approximately 100, 30, 10, 3, 1, 0.3, 0.1 and 0 mU/mL β-galactosidase standards. Add 50 µL/well of serially diluted β-galactosidase standards into a 96-well solid back plate.
Note 1: Adjust the standard curve (concentration range) to suit the specific experimental conditions, such as cell type, number, transfection efficiency, 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 assay mixture (from Step 1.3) to each well. Incubate the plate at room temperature or 37 °C for approximately 10 minutes to 4 hours depending on the cell type.

4.5 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.

4.6 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 plate reader.

Data Analysis

The absorbance in blank wells (with the assay buffer or lysis buffer mixed with assay mixture) is used as a control, and is subtracted from the values of those wells with cell extracts or β-galactosidase standards. The background of the blank wells varies depending upon the sources of the microtiter plates. A β-galactosidase (*E. coli*) standard curve is shown in Figure 1.

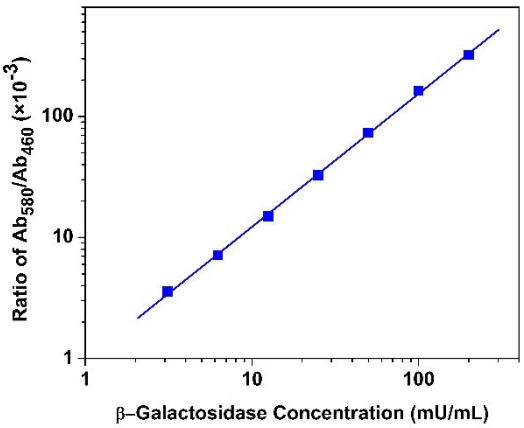

Figure 1. β-galactosidase dose response was measured with Amplite™ Colorimetric β-Galactosidase Assay Kit (Cat #: 12604) in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices). As low as 3 mU/mL β-galactosidase was detected with 30-60 minutes incubation. *(Note: The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

References

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