

ab324121 – Beta-Galactosidase Assay Kit (Colorimetric)

Can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324121

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Resorufin beta-D-Galactoside	1 vial	-20°C (Store in the dark)
Reaction Buffer	1 bottle (20 mL)	+4°C (Store in the dark)
Stop Buffer	1 vial (10 mL)	-20°C (Store in the dark)
Lysis Buffer	1 vial (10 mL)	-20°C (Store in the dark)
DMSO	1 vial (100 µL)	-20°C
beta-Mercaptoethanol	1 vial (100 µL)	-20°C (Store in the dark)

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Absorbance microplate reader (580/460 nm)
- Clear bottom plates

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 (Ab₅₈₀/Ab₄₆₀).

IMPORTANT: Thaw all kit components at room temperature before starting the experiment.

Preparation of Stock Solution

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.

Resorufin beta-D-Galactosidase stock solution (200X)

1. Add 50 µL of DMSO into the vial of Resorufin beta-D-Galactosidase to make 200X Resorufin beta-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

Beta-Galactosidase Standard solution

1. Optional (if a standard curve is desired): Prepare a serial dilution of beta-galactosidase (E. Coli) standards with 0.3% beta-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 beta-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 efficiency, 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 Solutions

0.3 % beta-mercaptoethanol assay buffer

1. Add 30 µL of beta-mercaptoethanol to 10 mL of Reaction Buffer, and mix well.

Note: Additional buffer is needed for preparing enzyme dilution buffer, which is used to generate a standard curve.

Resorufin betaDG working solution

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

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

Lysis buffer working solution

1. Add 5 µL of beta-mercaptoethanol to 5 mL of Lysis Buffer before use.

Note: Always add 0.1% beta-mercaptoethanol into lysis buffer before lysing the cells.

Experimental Protocol

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

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

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 beta-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 beta-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 µL/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 betaDG 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 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.

Data Analysis

The reading ($Ab_{580}/Ab_{460} \times 10^{-3}$) 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.

For technical support contact information, visit: www.abcam.com/contactus

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