

ab287846 – Beta Galactosidase Assay Kit (Green)

For the quantitative measurement of Beta Galactosidase.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab287846>

Storage and Stability

The entire kit may be stored at -20°C protected from light for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Fluorescence Standard IV	50 µL	-20°C
β-Gal Assay Buffer	25 mL	-20°C
β-Galactosidase	1 vial	-20°C
β-Gal Substrate	200 µL	-20°C

Materials Required, Not Supplied

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

- 96-well black plate with flat bottom is preferred for this assay, clear plate can also be used.
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

β-Gal Assay Buffer: Warm to room temperature before use. Store at 4°C or -20°C

β-Gal Substrate and Fluorescence Standard IV: Warm to room temperature before use

β-Galactosidase: Reconstitute with 100 µL Assay Buffer and mix thoroughly. Divide into aliquots. Keep on ice while in use. Use within 2 months.

Assay Protocol

Sample preparation

- 1) Rapidly homogenize tissue (~5 mg), cells (~1 x 10⁶) or yogurt (~5 mg) with 100 µl ice cold β-Gal Assay Buffer. Keep on ice for 10 min.
- 2) Centrifuge at 10,000 X g, 4 °C for 5 min. and collect supernatant.
- 3) Add 2-50 µl supernatant into desired well.
- 4) For Positive Control, dilute the β-Galactosidase/β-Gal Positive Control 1:25 by adding 10 µl of β-Galactosidase into 240 µl β-Gal Assay Buffer. Mix well. Add 1-20 µl of diluted β-Galactosidase into desired well(s). Adjust the volume of sample & Positive control wells to 50 µl/well with β-Gal Assay Buffer.

Δ Note: For Unknown Samples, we suggest doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range. For samples having background, prepare parallel sample well(s) as sample background control(s) and adjust the volume to 100 µl/well with β-Gal Assay Buffer.

Standard curve:

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- 1) Add 5 µl of 1 mM Fluorescein Standard to 995 µl of β-Gal Assay Buffer to generate 5 µM Fluorescein Standard Solution. Mix well.
- 2) Add 0, 2, 4, 6, 8 and 10 µl of 5 µM Fluorescence Standard IV into a series of wells in a 96-well plate to generate 0, 10, 20, 30, 40 and 50 pmol/well of Florescent Standard. Adjust the volume to 100 µl with Assay Buffer.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed, immediately before adding to the plate.
2. Prepare just enough for the number of reactions being run immediately.
3. Keeps the vial containing the reaction mix on ice while adding it to the well of the 96 well plate. For each well, prepare 50 µl Mix containing:

	Reaction Mix
β-Gal Assay Buffer	48 µl
β-Gal Substrate	2 µl

Mix well and add 50 µl of Reaction Mix into each Positive Control and sample well.

Δ Note: Don't add Reaction Mix to the Standard and sample background control wells.

Measurement

Measure the fluorescence (Ex/Em = 480/530 nm) immediately in kinetic mode for 5-30 min. at 37 °C.

Δ Note: We recommend measuring fluorescence in kinetic mode, and choosing two time points (T1 & T2) in the linear range to calculate the β-Gal activity of the samples. The Fluorescein Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

Calculation

- Subtract 0 Standard reading from all readings. Plot the Fluorescein Standard Curve.
- If the sample background control reading is significant, subtract the sample background control reading from sample reading.
- Calculate the Beta Galactosidase activity of the test sample:
 $\Delta\text{RFU} = \text{RFU}_2 - \text{RFU}_1$
- Apply ΔRFU to the Fluorescein Standard Curve to get B pmol of Fluorescein generated by Beta Galactosidase during the reaction time ($\Delta T = T_2 - T_1$).

Sample β-Galactosidase Activity = $B/(\Delta T \times V) \times \text{Dilution Factor} = \text{pmol}/\text{min}/\mu\text{l} = \mu\text{U}/\mu\text{l} = \text{mU}/\text{ml}$

Where: B is Fluorescein amount in the sample well from Standard Curve (pmol)

ΔT is reaction time (min.)

V is sample volume added into the reaction well (µl)

Beta Galactosidase Activity in samples can also be expressed as µU/mg of protein.

Unit Definition: One unit of β Galactosidase is the amount of enzyme that generates 1.0 µmol of Fluorescein per min. at pH 7.0 at 37 °C

Technical Support

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