Beta Galactosidase (β-Gal) Inhibitor Screening Kit (Fluorometric) (#BN01044)

(Catalog # BN01044; 100 assays; Store at -20°C)

I. Introduction:

Beta Galactosidase (β-Gal, EC: 3.2.1.23) is an enzyme which hydrolyzes the β-galactosides into monosacchanides. β-Gal is widely used as a reporter gene in the field of molecular biology. Senescence Associated β-Gal (SA-β-Gal) is an isoform of β-Gal which has the optimal activity at pH 6.0, and is mostly used as a biomarker for senescent cells (K802). β-Gal is an essential enzyme in humans and its deficiency results in Morquio's Syndrome, a severe birth defect. β-Gal can also be used as a tool to study protein-protein interaction. In Assay Genie's Beta-Galactocidase Inhibitor Screening Kit, β-Gal converts β-Gal substrate to give an intensely fluorescent product (Ex/Em = 480/520 nm). In the presence of a β-Gal inhibitor, the reaction is impeded/abolished resulting in decrease or total loss of fluorescence. This assay kit can be used to screen/study/characterize the potential inhibitors of Beta Galactosidase. The assay is simple, high-throughput adaptable and can be performed within 30 min.

β-Gal Substrate-Fluorescein $\xrightarrow{\text{β-Galactosidase}}$ Galactose + Fluorescence (Ex/Em = 480/520 nm)

II. Application:

- Screening/characterizing/studying potential inhibitors of Beta-Galactosidase.

III. Kit Contents:

Components	BN01044	Cap Color	Part Number
β-Gal Assay Buffer	25 ml	WM	BN01044-1
β-Gal Substrate (in DMSO)	200 µl	Blue	BN01044-2
β-Galactosidase	1 vial	Purple	BN01044-3
β-Gal Inhibitor Control	1 vial	Orange	BN01044-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **β-Gal Assay Buffer:** Bring to room temperature before use. Store at -20°C or 4°C.
- **β-Gal Substrate:** Thaw at room temperature. Aliquot and store at -20°C.
- **β-Galactosidase:** Reconstitute with 550 µl β-Gal Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **β-Gal Inhibitor Control:** Reconstitute with 200 µl dH_2O. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

VI. β-Gal Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at 100X the final concentration to be tested. Dilute to 2X desired test concentration with β-Gal Assay Buffer. Add 50 µl diluted candidate inhibitor or β-Gal Assay Buffer into desired wells, as Sample [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), dilute Inhibitor Control 5 times by adding 20 µl Inhibitor Control to 80 µl β-Gal Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s).

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well(s) with the same final concentration of the solvent(s) as in the inhibitor sample(s) as solvent controls (SC).
2. **β-Gal Enzyme:** Add 5 µl β-Gal Enzyme into Sample, Enzyme Control, and Inhibitor Control wells (if necessary, in Solvent Control wells). Incubate for 5 min. at 25°C. Add 55 µl of Assay Buffer into separate well designated as BC (Background Control).
3. **Substrate Solution Preparation**: Make enough reagents for the number of assays to be performed. For each well, prepare 45 µl of Substrate solution containing:

β-Gal Assay Buffer	43 µl
β-Gal Substrate	2 µl

   Mix and add 45 µl of Substrate solution into each well. Mix well with gentle shaking, protected from light.
4. **Measurement:** Measure fluorescence (Ex/Em = 480/520 nm) in kinetic mode for 5-30 min. at 37°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 & RFU_2).
5. **Calculations:** Subtract from all samples the background (ΔBC = BC_2-BC_1). Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the corrected ΔRFU (RFU_2-RFU_1) values with the time ΔT (T_2-T_1).

$$\%\ \textbf{Relative Inhibiton} = \frac{\textbf{(Slope of EC} - \textbf{Slope of S)}}{\textbf{Slope of EC}}\ \textbf{X 100}$$

Notes:

a. Irreversible inhibitors that inhibit the β-Gal activity completely at the tested concentration will have ΔRFU = 0 and thus the % Relative Inhibition will be 100%.

b. In case Solvent Control(s) has substantially different slope(s) than the EC, use SC slope(s) instead of Slope of EC in the equation above.

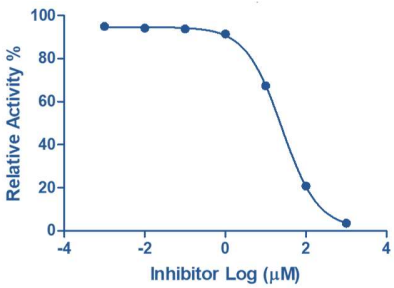

Figure: Inhibition of Beta-Galactosidase activity by β-Gal Inhibitor (b-D-Galactopyranosyl Amine). IC_{50} = 25.30 µM. Assay was performed following the kit protocol.

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