Beta Galactosidase (β-Gal) Activity Assay Kit (Fluorometric) (#BN01038)

(Catalog # BN01038; 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 (#BN01018). β-Gal is an essential enzyme in humans and its deficiency results in Morquio's Syndrome, a severe birth defect. Assay Genie's Beta Galactosidase Activity Assay kit provides a quick and easy way for monitoring Beta Galactosidase activity in a variety of samples. In this kit, Beta Galactosidase hydrolyses a non-fluorescent substrate to generate a strong fluorescent product. The assay is simple, sensitive, and high-throughput adaptable. Detection limit: < 0.1 mU.

II. Application:

- Measurement of β-Galactosidase activity in various samples

III. Sample Type:

- Prokaryote such as: *E.coli*
- Animal tissues such as spleen etc.
- Adherent or suspension cells
- Food such as yogurt
- White blood cells

IV. Kit Contents:

Components	BN01038	Cap Code	Part Number
β-Gal Assay Buffer	25 ml	WM	BN01038-1
β-Gal Substrate (in DMSO)	200 µl	Blue	BN01038-2
Fluorescein Standard (1 mM)	50 µl	Yellow	BN01038-3
β-Gal Positive Control	1 vial	Purple	BN01038-4

V. User Supplied Reagents and Equipment:

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

VI. 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:** Warm to room temperature before use. Store at 4°C or -20°C.
- **β-Gal Substrate and Fluorescein Standard:** Thaw at room temperature. Store at -20°C.
- **β-Gal Positive Control:** Reconstitute with 100 µl β-Gal Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. β-Gal Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (~5 mg), cells (~1 x 10^6) or yogurt (~5 mg) with 100 µl ice cold β-Gal Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g, 4°C for 5 min. and collect supernatant. Add 2-50 µl supernatant into desired well. For Positive Control, dilute the β-Gal Positive Control 1:25 by adding 10 µl of β-Gal Positive Control into 240 µl Assay Buffer. Mix well. Add 1-20 µl of diluted β-Gal Positive Control into desired well(s). Adjust the volume of sample & Positive control wells to 50 µl/well with β-Gal Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. 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.

   c. We recommend diluting the β-Gal Positive Control just before use. Don't store the diluted β-Gal Positive Control.

2. **Standard Curve:** 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. Add 0, 2, 4, 6, 8 and 10 µl of 5 µM Fluorescein Standard into a series of wells in a 96-well plate to generate 0, 10, 20, 30, 40 and 50 pmol/well of Fluorescein Standard. Adjust the volume to 100 µl with β-Gal Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction 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.

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

4. **Measurement:** Measure fluorescence (Ex/Em = 480/520 nm) immediately in kinetic mode for 5-30 min. at 37°C.

   Note: Incubation time depends on the β-Gal activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 & T_2) 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).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Fluorescein Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading.

   Calculate the Beta Galactosidase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. 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/(ΔT X V) x Dilution Factor = pmol/min/µl = µU/µl = mU/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.

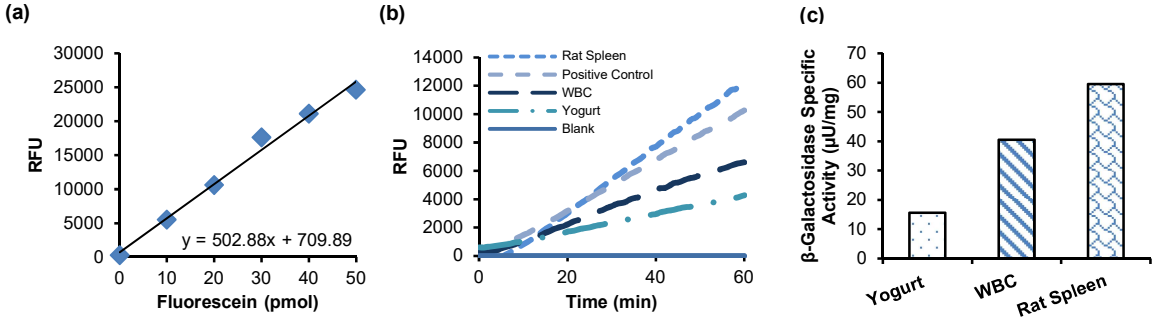

Figure: (a) Fluorescein Standard Curve, (b) Measurement of Beta Galactosidase activity in Yogurt (8 µg), rat spleen (8 µg), and WBC (6 µg) homogenate and (c) Beta Galactosidase specific activity of samples mentioned in Fig. b. Assay was performed according to the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.