BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

LacZ Assay Kit (Cat #: E-104)

COMPONENTS: LacZ Assay Solution- 20 ml, store at -20ºC (for 200 wells)
LacZ Stop Solution- 10 ml, store at room temperature
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The bacterial enzyme β-galactosidase (LacZ) is a widely used reporter enzyme, which can be measured spectrophotometrically (J. Med. Chem. 7:574,1964). The assay utilizes o-nitrophenyl-β-D-galactopyranoside (ONPG) for measurement of LacZ activity in eukaryotes and prokaryotes transfected with a LacZ reporter DNA. LacZ converts ONPG to o-nitrophenol, which exhibits an extinction coefficient of 18 $mM^{-1}cm^{-1}$ at 405 nm. Cell Lysis Solution is compatible with luciferase and CAT enzyme assays. Kit components are stable for at least one year if handled and stored properly.

Preparation of animal cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 100 – 200 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 2 mg/ml.

LacZ assay for animal cell/tissue:

1. Thaw LacZ Assay Solution and keep on ice during assay. Add 10 µl of each sample to a plain (uncoated) 96-well microplate. Use 10 µl 1x Cell Lysis Solution or dH_2O as blank. Reaction is initiated by adding 100 µl LacZ Assay Solution to each well. Mix contents by brief gentle agitation. Cover the plate, and incubate at 37°C for 30 or 60 min (do not use CO_2 incubator).

2. Terminate reaction by adding 50 µl LacZ Stop Solution per well followed by brief gentle agitation. Measure O.D. at 405 nm using a microplate reader. Subtract blank well reading from all sample well readings to obtain **ΔO.D.**.

LacZ assay for yeast/bacteria:

1. Pellet 1 – 2 ml yeast or bacterial culture in a microtube by a 20-sec spin in a microfuge at ~14,000 rpm. Aspirate off medium. Add 100 µl LacZ Assay Solution to the cell pellet and vortex vigorously to resuspend cells.

2. Add 50 µl chloroform to the mix followed by 1 min vortexing. Incubate reaction at 37°C for 30 or 60 min (do not use CO_2 incubator).

3. Terminate reaction by adding 50 µl LacZ Stop Solution per tube, vortex tube briefly, and spin tube for 3 min. Transfer 160 µl of the upper layer to a microplate, and measure O.D. at 405 nm.

Calculation of enzyme activity:

For 30 min incubation, LacZ activity in IU/L = µmol/(L•min) = **ΔO.D.** x 1000 x 160 µl / (30 min x 0.8 cm x 18 x 10 µl) = **ΔO.D. x 37.04**. If incubation for 60 min, LacZ activity **= ΔO.D. x 18.52**. Sample enzyme activity can be presented as units/µg proteins.

Additional information:

- Chloroform is highly volatile, and should be handled in the fume hood. Avoid skin contact. Please refer to the product page of our website or contact us for MSDS information.