BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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β-Galactosidase Assay kit (Cat #: E-150)

COMPONENTS: β-Galactosidase Assay Solution- 10 ml (for 100 wells), store at -20ºC
β-Galactosidase Control Solution- 10 ml, store at -20ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The human GLB1 gene encodes two forms of beta-galactosidase. Isoform 1 is a lysosomal enzyme responsible for cleaving beta-linked terminal galactosyl residues from gangliosides, glycoproteins, and glycosaminoglycans. The assay is based on the cleavage of nitrophenyl from the substrate 2-nitrophenyl-β-D-galactopyranoside, which increases absorbance at 405 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), allowing for measurement of enzyme activity present in tissue/cell lysates and serum. Kit components are stable for at least 1 year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~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 0.2 – 2 mg/ml.

Enzyme assay for cell/tissue lysates:

1. Thaw Assay Solution and keep solution on ice. *Do not over thaw.* Add 20 µl of each cell/tissue sample to a plain (uncoated) 96-well plate. Add 20 µl 1x Cell Lysis Solution to an empty well serving as blank.
2. Reaction is initiated by adding 100 µl β-Galactosidase Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min. *Do not use CO_2 incubator.*
3. Measure $O.D._{405\ nm}$ using a plate reader. Subtract blank well reading from each sample well reading to generate **ΔO.D.** (see below for enzyme activity calculation).

Enzyme assay for serum:

1. Thaw Assay Solution and Control Solution and keep solutions on ice. Do not over thaw. Add 5 µl of each serum sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).
2. Add 100 µl β-Galactosidase Control Solution to one set of sample wells (control). Add 100 µl β-Galactosidase Assay Solution to the other set of sample wells (reaction). Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min. *Do not use CO_2 incubator*.
3. Measure $O.D._{405\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to generate **ΔO.D.** for each sample.

Enzyme activity calculation:

For 20 µl sample, β-Galactosidase enzyme activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 120 µl / (60 min x 0.6 cm x 18 x 20 µl) = **ΔO.D. x 9.26**.

For 5 µl sample, β-Galactosidase enzyme activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 105 µl / (60 min x 0.6 cm x 18 x 5 µl) = **ΔO.D. x 32.41**.

Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- Please refer to the product page of our website or contact us for SDS information.
- 10x Cell Lysis Solution precipitates upon freezing. Re-dissolve contents by agitation upon arrival.