BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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α-Galactosidase Assay kit (Cat #: E-149)

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

PRODUCT DESCRIPTION: The lysosomal α-galactosidase (α-GAL) breaks down a fatty substance designated as globotriaosylceramide. Failure to turn over the sphingolipid causes accumulation of globotriaosylceramide in the lysosomes, causing Fabry disease. The assay is based on cleavage of nitrophenyl from the substrate 4-nitrophenyl-α-D-galactopyranoside, which increases absorbance at 405 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), allowing for measurement of α-GAL 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 at least ~10^5 washed cells in 50 – 100 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 1 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Enzyme assay for cell/tissue lysates:

1. Thaw α-GAL Assay Solution and keep solution on ice (do not over-thaw). Add 20 µl of each 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 α-Gal Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min or 90 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.**

Enzyme assay for serum:

1. Thaw α-GAL Assay Solution and Control Solution and keep both 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 assay solution).
2. Add 100 µl α-GAL Control Solution to one set of sample wells. Add 100 µl α-GAL Assay Solution to the other set of sample wells. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 60 min or 90 min. *Do not use CO_2 incubator.*
3. Measure $O.D._{405\ nm}$ using a plate reader. Subtract control well reading from assay well reading to generate **ΔO.D.** for each sample.

Enzyme activity calculation:

For 20 µl sample, α-GAL 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, α-GAL 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.

Additional information:

- Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.