BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Acid α-Glucosidase (GAA) Assay kit (Cat #: E-148)

COMPONENTS: GAA Assay Solution- 10 ml (for 100 wells), store at -20ºC
GAA 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 lysosomal acid α-glucosidase (GAA or acid maltase) assay is based on the hydrolysis of 4-nitrophenyl-α-D-glucopyranoside. The enzyme is capable of degrading glucose-α-1,4-glucose and glucose-α-1,6-glucose glycosidic bonds. Cleavage of nitrophenyl from the substrate increases absorbance at 405 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), allowing for measurement of GAA 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 -70ºC.
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.* Slight precipitates may appear after thawing. Agitate container to dissolve contents if necessary. 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 GAA 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.**.

Enzyme assay for serum:

1. Thaw Assay Solution and Control Solution and keep solutions on ice. *Do not over thaw.* Slight precipitates may appear after thawing. Vortex container to dissolve contents if necessary. Add 5 µl of each serum sample to duplicate wells of a plain (uncoated) 96-well plate (for control set and reaction set).
2. Add 100 µl GAA Control Solution to one set of sample wells (control). Add 100 µl GAA 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, GAA 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, GAA 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 in warm water upon arrival.