BIOMEDICAL RESEARCH SERVICE CENTER
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O-GlcNAcase (OGA) Assay Kit (Cat #: E-130)

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

PRODUCT DESCRIPTION: The OGA assay is based on the cleavage of p-nitrophenyl-β-N-acetyl-glucosaminide to nitrophenol, the ionization of which by NaOH produces a yellow color exhibiting an absorption maximum at 405 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), which allows for sensitive detection of OGA activity present in crude cell/tissue lysates and biological fluids such as serum/plasma. Kit components are stable for at least one 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 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 1 – 2 mg/ml.

Enzyme assay for clear sample:

1. Thaw OGA Assay Solution and keep solution on ice during assay. Add 10 µl of each sample to a plain (uncoated) 96-well plate. Add 10 µl 1x Cell Lysis Solution or dH_2O to an empty well serving as blank.
2. Reaction is initiated by adding 100 µl OGA Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 30 min or 60 min (do not use CO_2 incubator).
3. Stop reaction by adding 20 µl 1N NaOH (not included in the kit) to each well followed by brief gentle agitation. Yellowish reaction color caused by enzyme activity will appear upon NaOH addition.
4. 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 colored sample (serum/plasma):

1. Thaw OGA Assay Solution. Keep both OGA Assay Solution and OGA Control Solution on ice during assay. Add 10 µl of each sample to a plain (uncoated) 96-well plate <u>in duplicate</u> for control and assay wells.
2. Add 100 µl OGA Control Solution to one set of sample wells. Add 100 µl OGA Assay Solution to the other set of sample wells. Mix contents by brief agitation. Cover plate and incubate at 37°C for 30 min or 60 min (do not use CO_2 incubator).
3. Stop reaction by adding 20 µl 1N NaOH (not included in the kit) to each control well and assay well followed by brief agitation. Yellowish reaction color will appear upon NaOH addition.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract control well reading from assay well reading for each sample to generate **ΔO.D.**.

Enzyme activity calculation:

For 30 min incubation, OGA enzyme activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 130 µl / (30 min x 0.6 cm x 18 x 10 µl) = **ΔO.D. x 40.12**. For 60 min incubation, OGA enzyme activity **= ΔO.D. x 20.06**. For cell/tissue lysates, sample enzyme activity can be presented as units/µg proteins.

Additional information:

- OGA Assay Solution and Control Solution contain acetic acid and sodium acetate. Avoid skin contact.
- A solution of 1N NaOH needs to be prepared by end users for reaction termination. Avoid skin contact with NaOH. Please refer to the product page of our website or contact us for MSDS information.