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

COMPONENTS: OGA Assay Solution- 20 ml (200 assays), **store in aliquots at -80ºC after the first thawing**
OGA Control Solution- 10 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: The OGA assay is based on the cleavage of the artificial substrate p-nitrophenyl-β-N-acetyl-glucosaminide to nitrophenol in an acidic buffer. Ionization of nitrophenol by NaOH produces a yellow color exhibiting an absorption maximum at 405-415 nm (ε = 18.75 $mM^{-1}cm^{-1}$), which allows for sensitive detection of OGA activity in crude cell/tissue extracts, serum/plasma and urine. Repeated freeze-thaw cycles of OGA Assay Solution should be avoided.

Preparation of cell/tissue extracts:

1. Wash 10^5 – 10^6 cells twice with ice-cold phosphate-buffered saline (PBS), and remove PBS completely from the cell pellet. Cell pellet should be stored at -80ºC. Tissue sample should be washed with PBS thoroughly to remove blood cells, which can cause inconsistent assay result.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. If lysate is viscous, add more 1x Cell Lysis Solution and repeat pipetting. Centrifuge lysate in a refrigerated microfuge for 3 min at maximal speed. Recover supernatant for assay. Tissue is homogenized in 1x Cell Lysis Solution, and lysate is clarified by centrifugation. Use ~25 mg of tissue per 0.5 ml of 1x Cell Lysis Solution for tissue homogenization.
3. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution to 0.5 – 1 mg/ml. Keep protein sample on ice at all times. Freeze-thawed crude protein lysate can exhibit reduced enzyme activity. Lysate should be stored at -80ºC. Note: Do not use a buffer containing reducing agents or SDS.

Enzyme assay for clear sample: Thaw OGA Assay Solution and keep solution on ice prior to assay.

1. Add 10 µl sample to each well of a 96-well plate. Add 10 µl dH_2O to a 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.
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 **O.D.**. For 30 min incubation, OGA enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 130 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 38.52**. For 60 min incubation, OGA activity in IU/L unit **= O.D. x 19.26.**

Enzyme assay for hemolyzed sample:

1. Add 10 µl sample to each well of a 96-well plate in duplicate.
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.
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 **O.D.**. For 30 min incubation, OGA enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 130 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 38.52**. For 60 min incubation, OGA activity in IU/L unit **= O.D. x 19.26.**

Additional information:

- Note that "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- OGA Assay Solution and Control Solution contain acetic acid. Avoid skin contact.
- A 1N NaOH solution needs to be prepared for reaction termination. Avoid skin contact with NaOH. Please refer to the product page of our website or contact us for MSDS information.