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O-GlcNAcase (OGA) Assay Kit (Cat #: E-130)

COMPONENTS: OGA Assay Solution- 10 ml (for 100 wells), store at -20ºC
OGA Control Solution- 10 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

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. 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 dH_2O. Bring up ~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 (~5 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 0.5 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Enzyme assay for clear sample:

1. Thaw OGA Assay Solution and keep solution on ice during assay. Add 20 µl of each sample to a plain (uncoated) 96-well plate. Add 20 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. Keep plate on bench for 3 min prior to optical measurement.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract blank well reading from each sample well reading to generate **ΔO.D.**. For 30 min incubation, OGA activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 140 µl/(30 min x 0.6 cm x 18 x 20 µl) = **ΔO.D. x 21.6** (or **ΔO.D. x 10.8** for 60 min). Cell/tissue OGA activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity.

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 duplicate wells of a plain (uncoated) 96-well plate (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. Keep plate on bench for 3 min prior to optical measurement.
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.** For 30 min incubation, OGA activity = 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** (or **ΔO.D. x 20.06** for 60 min).

Additional information:

- OGA Assay Solution and Control Solution contain acetic acid and sodium acetate. Avoid skin contact.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.
- Refer to the product webpage or contact us for SDS information.