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Nagalase Assay Kit (Cat #: E-142)

COMPONENTS: Nagalase Assay Solution- 5 ml (for 100 wells), store at -20ºC
Nagalase Control Solution- 5 ml, store at room temperature
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**
NaOH Stop- 5 ml, store at room temperature (**caution: avoid skin contact**)

PRODUCT DESCRIPTION: The Nagalase assay is based on the cleavage of the artificial substrate p-nitrophenyl-α-N-acetyl-galactosaminide to nitrophenol in an acidic buffer. Ionization of nitrophenol by NaOH produces a yellow color exhibiting an absorption maximum at 405 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$), which allows for measurement of Nagalase activity in crude cell/tissue extracts and biological fluids such as serum.

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 lysate protein concentration range is 0.5 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Enzyme assay for clear sample: Thaw Nagalase Assay Solution and keep solution on ice during assay.

1. Add 10 µl sample to a plain (uncoated) 96-well plate. Add 10 µl dH_2O to a well serving as blank.
2. Reaction is initiated by adding 50 µl Nagalase 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. Stop reaction by adding 20 µl NaOH Stop to each well followed by brief gentle agitation. Keep plate on bench (room temperature) 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 obtain **ΔO.D.**. Nagalase enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** x 1000 x 80 µl/(60 min x 0.4 cm x 18 x 10 µl) = **O.D. x 18.52.** Cell/tissue nagalase activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Enzyme assay for colored sample, e.g. serum:

1. Add 10 µl sample to duplicate wells of a plain (uncoated) 96-well plate (for control and assay wells).
2. Add 50 µl Nagalase Control Solution to one set of sample wells. Add 50 µl Nagalase Assay Solution to the other set of sample wells. Mix contents by brief agitation. Cover plate and incubate at 37°C for 60 min. *Do not use CO_2 incubator.*
3. Stop reaction by adding 20 µl NaOH Stop to each well followed by brief gentle agitation. Keep plate on bench (room temperature) 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.**. Nagalase enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** x 1000 x 80 µl/(60 min x 0.4 cm x 18 x 10 µl) = **ΔO.D. x 18.52**.

Additional information:

- Nagalase Assay Solution and Control Solution contain a dilute solution of acetic acid. Avoid skin contact.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.
- NaOH Stop contains a dilute solution of sodium hydroxide. Avoid skin contact. Please refer to the product webpage or contact us for SDS information.