BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Nagalase Assay Kit (Cat #: E-142)

COMPONENTS: Nagalase Assay Solution- 5 ml (100 wells), **store in aliquots at -80ºC after the first thawing**
Nagalase Control Solution- 5 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

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 405-415 nm (molar extinction coefficient = 18.75 $mM^{-1}cm^{-1}$), which allows for sensitive detection of Nagalase activity in crude cell/tissue extracts, serum/plasma and urine.

Preparation of cell/tissue extracts:

1 Wash ~10^6 cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80ºC until use.

2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl 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. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm) and harvest supernatant for OGA assay. For tissue extraction, weigh ~25 mg tissue and homogenize in 0.5 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4ºC for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80ºC.

3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.5 – 2 mg/ml.

Enzyme assay for clear sample: Thaw Nagalase 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 50 µl Nagalase 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, Nagalase enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 80 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 23.7**. For 60 min incubation, Nagalase activity in IU/L unit **= O.D. x 11.85.**

Enzyme assay for hemolyzed sample:

1. Add 10 µl sample to each well of a 96-well plate in duplicate.

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 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, Nagalase enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 80 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 23.7**. For 60 min incubation, Nagalase activity in IU/L unit **= O.D. x 11.85.**

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.
- Nagalase 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.