Tribioscience

Beta- N-Acetylglucosaminidase (Beta - NAG) Activity Fluorometric Assay (Store at -20°C)

Catalog Number	Kit Size
TBS2105F-100	100 assays
TBS2105F-200	200 assays

Description

β-N-Acetylglucosaminidase (β-NAG, β-Hexosaminidase, EC 3.2.1.52) is a lysosomal enzyme that is expressed in various tissues, including kidneys, liver, lungs, and mast cells. Elevated enzyme levels are associated with many disorders, such as Tay-Sachs and Sandhoff disease, inflammation, abnormal immune responses.

The β-N-Acetylglucosaminidase Activity Fluorometric Assay provides a simple, accurate, and sensitive method for monitoring N-acetylglucosaminidase activity in biological samples (tissue, cells, serum, urine). This assay uses a non-fluorescent substrate 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MUAG) to release 4-Methylumbelliferone (4-MU) which can be measured at an excitation wavelength of 365 nm and an emission wavelength 445 nm.

Applications

This kit is used for the determination of hexosaminidase activity in biological samples.

Key features

Fast and sensitive: Linear detection range (20 µL sample) for a 30 minutes reaction at 37˚C

High throughput*:* Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Kit Contents

Component	100x Rxns	200x Rxns
Substrate (100X)	100 µL	200 µL
Standard (1 mM)	50 µL	100 µL
NAG positive control	50 µL	100 µL
Stop Reagent	12 mL	24 mL
Assay Buffer	15 mL	30 mL

STORAGE CONDITIONS

The kit is shipped on ice and should be stored at -20°C for long-term storage. Shelf life of 12 months after receipt.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

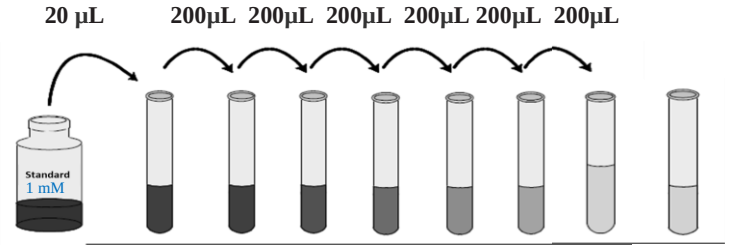
Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Collect the supernatant for assay.

Reagent Preparation: Equilibrate all components to 37°C. Briefly vortex or pipette up and down all components to ensure fresh reconstitution.

Reaction Preparation:

1. Prepare the substrate working solution by diluting 100-fold of the stock, eg add 9.9 mL assay buffer to 100 µL of substrate.
2. Label tubes as #1 through #8 as below diagram.
3. Add 380 µL of 1x Assay Buffer to Std1, and 200 µL of 1xAssay Buffer to Std2 to 8.
4. Pipet 20 µL of 1 mM standard stock into Std#1. Then, then make 2x series dilution in Std2 through 7 with addition of 200 µL. Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration in tube 1 through 7 will be 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µM, Tube#8 is Standard 0 as blank.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	380	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	20	200	200	200	200	200	200	0
Final Conc (µM)	50	25	12.5	6.25	3.125	1.56	0.78	0

5. Transfer 20 µL of each sample, blank, positive control and standards into two separate wells of a black 96-well plate.
6. Add 80 µL of the substrate working solution to the sample, positive control, and blank wells. Add 80 µL of the assay buffer to the standards. *(Note: Do not add substrate in the standard*). Tap plate briefly to mix.
7. Incubate at 37°C or desired temperature for 30 minutes.
8. Add 100 µL of Stop Reagent to all wells. Tap plate briefly to mix.
9. Read at Ex/Em = 365 nm/445 nm.

CALCULATION

Subtract blank RFU (Standard 0, #8) from the standard RFU values and plot the ΔRFU against standard concentrations. Determine the slope, and use the following equation to calculate NAGase activity:

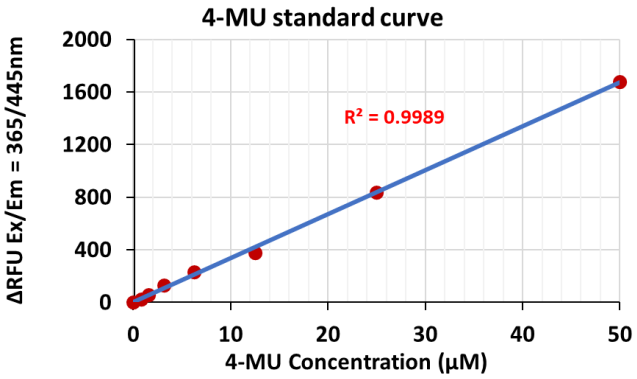
NAG Activity (μM) = DF * (RFU SAMPLE – RFU BLANK)/ (t * Slope)

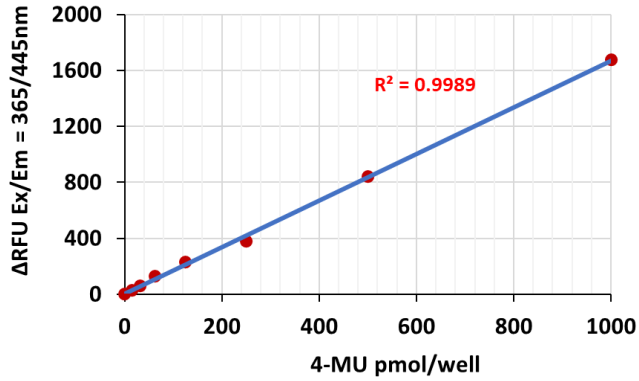
where RFU SAMPLE is the measurement for each sample and RFU BLANK is the fluorescent value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 μmole of 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MUAG) to 4-Methylumbelliferone (4-MU) and N-acetyl-D glucosamine per min at 37°C and at 0pH 4.5

TYPICAL DATA

This standard curve is provided for demonstration only as below figure. A standard curve should be generated for each set of samples assayed.





RELATED PRODUCTS

LDH Cytotoxicity colorimetric Assay (TBS2002)
Tryptase Activity Assay (TBS2101)
B-N-acetylglucosaminidase Activity Colorimetric Assay (TBS2105)
Caspase-3 Fluorometric Assay kit (TBS3230)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

Research use only.