Tribioscience

Beta-Hexosaminidase Activity Colorimetric Assay

Catalog Number	Kit Size
TBS2105-100	100 assays
TBS2105-200	200 assays

Description

β-Hexosaminidase (β-N-Acetylglucosaminidase, NAGase, 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, and abnormal immune responses.

The Beta-Hexosaminidase Activity Colorimetric Assay provides a simple and sensitive method for monitoring hexosaminidase activity in biological samples (tissue, cells, serum, urine). This assay uses a synthetic p-nitrophenol derivative (R-*p*NP) as its substrate and releases *p*NP which can be measured at absorbance (OD 405 nm). The assay can detect as low as 50 µU of NAGase activity in a variety of samples.

Applications

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

Key features

Fast and sensitive: Linear detection range (20 µL sample): 0.05 to 50 U/L for a 30-minute 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	10 mL	20 mL
Standard (10mM)	1 mL	2 mL
NAG positive control	50 µL	100 µL
Stop Reagent	12ml	24mL
Assay Buffer	12ml	24mL

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.

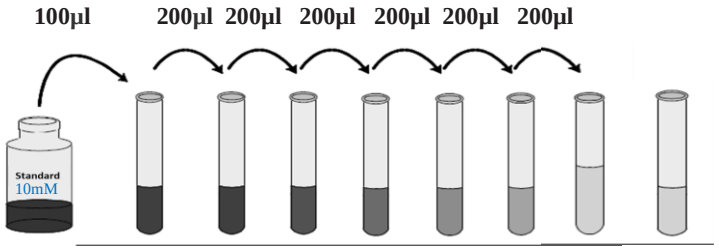
Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000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

Reagent Preparation:

- Equilibrate all components to 37°C. If substrate has precipitation in bottle, please warm up to dissolve the precipitation.
- Briefly vortex or pipette up and down all components to ensure fresh reconstitution.

Reaction Preparation:

1. Label tubes as #1 through #8 as below diagram.
2. Add 400 µL of 1x Assay Buffer to Std1, and 200 µL to Std2 to 8.
3. Pipet 100 µL of 10 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 2000,1000, 500, 250, 125, 62.5 and 31.25µM, Tube#8 is Standard 0 as blank.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	400	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	100	200	200	200	200	200	200	0
Final Conc (µM)	2000	1000	500	250	125	62.5	31.25	0

4. Transfer 20 µL of each sample, blank, positive control, and standards into two separate wells.
5. Add 80 µL of the substrate solution to all sample, positive control, and blank wells. Add 80 µL of Assay Buffer to each standard well *(Note: Do not add substrate in the standard*). Tap plate briefly to mix.

3. Incubate at 37°C or desired temperature for 30-60 minutes.
4. Add 100 µL of Stop Reagent to all wells. Tap plate briefly to mix.
5. Incubate at 37°C for 20 minutes
6. Read OD405nm.

CALCULATION

Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the

slope, and use the following equation to calculate NAGase activity:

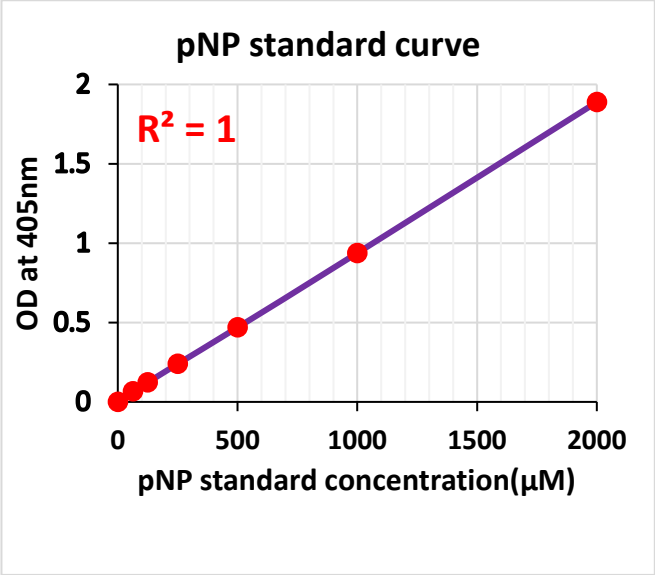
NAG Activity (U/L) = DF * (ODSAMPLE – OD BLANK)/ (t * Slope)

where ODSAMPLE is the OD405nm value for each sample and ODBLANK is the OD405nm 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 pNitrophenyl N-acetyl-β-D-glucosaminide to p-Nitrophenol
and N-acetyl-D glucosamine per min at 37°C and pH 4.5 (***Note:*** *If sample NAG activity exceeds 200 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with NAG activity <1U/L, the incubation time can be extended up to 4 hours for greater sensitivity*).

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:

Caspase-3 Fluorometric Assay kit (TBS3230)
Tryptase Activity Assay (TBS2101)
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.