101Bio.com Beta-Hexosaminidase Activity Colorimetric Assay (Cat. # T2105) Manual

Description

This kit is used for determination of hexosaminidase activity in biological samples.
β-Hexosaminidase (β-N-Acetylglucosaminidase, NAG, EC 3.2.1.52) is a lysosomal enzyme that is expressed in various tissues, including kidney, 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 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 NAG activity in a variety of samples. This product is for research use only.

Key features

- **Fast and sensitive**: Linear detection range (20 µL sample): 0.2 to 50 U/L for a 30 minute reaction at 37˚C.
- **High-throughput***:* it can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Kit Contents: this product is for 200 tests.
Assay Buffer: 1mL; Stop Reagent: 12 mL
Substrate: 10 mL; Standard: 1 mL (10 mM Nitrophenol)

CONDITIONS: Ship at 4°C , store at - 20°C, shelf life: 12 months

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. Briefly vortex or pipette up and down all components to ensure fresh reconstitution.
Standard Preparation: Mix 10 mM standard with Assay Buffer as the Table:

Standard	Standard stock(µL)	Assay Buffer(µL)	Volume (µL)	*p*NP (µM)
1	20	80	100	200
2	15	85	100	150
3	10	90	100	100
4	5	95	100	50
5	2.5	97.5	100	25
6	0	100	100	0

Reaction Preparation

1. Transfer 20 µL of each sample into two separate wells (ODSAMPLE and ODSAMPLE BLANK). Transfer 20 µL of each standard (ODSTD) into separate wells of a clear flat bottom 96-well plate.

2. Add 100 µL of stop reagent to the Sample Blank wells.
3.Add 80 µL of the substrate solution to all standard, sample, and sample blank wells. Tap plate briefly to mix.

3. Incubate at 37°C or desired temperature for 30 minutes.
4. Add 100 µL of Stop Reagent to each standard and sample well. **Note:** *Do not add anything more to the sample blank wells.* Tap plate briefly to mix.
5. Read OD405nm.

CALCULATION

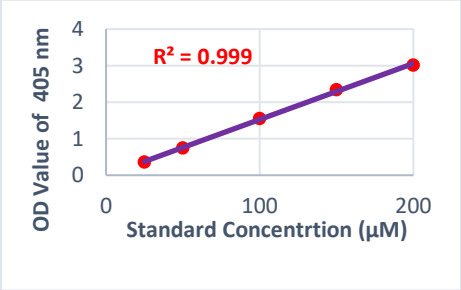
Subtract blank OD (assay buffer, #6) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate β-N-Acetylglucosaminidase activity:

NAG Activity (U/L) = DF x (ODSAMPLE – ODSAMPLE BLANK)/ t • Slope

where ODSAMPLE is the OD405nm value for each sample and ODSAMPLE BLANK 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-Dglucosamine 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 < 1 U/L, the incubation time can be extended up to 4 hours for greater sensitivity.

Standard Curve of pNP



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