PRODUCT INFORMATION & MANUAL

beta-N-acetyl-glucosaminidase/NAG Activity Assay Kit (Colorimetric)

NBP3-25920

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

beta-N-acetyl-glucosaminidase/NAG Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25920
Method: Colorimetric method
Specification: 50Assays **(**Can detect 24 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 0.1U/L
Detection range: 0.1-80U/L

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used for detection of beta-N-acetyl-glucosaminidase/NAG Activity in serum (plasma), urine, animal tissue sample.

Detection principle

beta-N-acetyl-glucosaminidase/NAG is an acid hydrolase in lysosome and exist wildly in various tissues, organs, body fluid, red blood cells, leukocytes and blood platelets. The substrate was hydrolyzed by the catalysis of NAG and release free p-nitrophenol. Adding alkaline solution to stop the reaction and triggers color reaction at the same time. The NAG activity can be calculated indirectly by measuring the OD value at 400 nm.

Kits components

Reagent	Component	Specification	Storage
Reagent 1	Liquid	40 mL × 1 vial	2-8℃, 12 months
Reagent 2	Substrate	Powder × 1vial	2-8℃, 12 months
Preparation of the substrate solution: The solubility of the substrate is low. Use appropriate amount of the reagent 1 to concoct the reagent 2 powder into mash. Slowly add the reagent 1 into the mesh while stirring until reach 30 mL. Mix thoroughly until the powder is fully dissolved. (Do not heat). The substrate solution is oversaturated solution. If crystalize, take the supernatant for experiment. The unused substrate solution can be stored at 2-8℃ for more than 2 month.			
Reagent 3	Stop Solution	60 mL × 2 vials	2-8℃, 12 months
Reagent 4	Liquid	6 mL × 1 vial	2-8℃, 12 months
Reagent 4 will crystallize out in cold weather and incubate in 37℃ water bath until transparent before use.			
Reagent 5	3 mmol/L p-Nitrophenol Standard Stock Solution	2 mL × 1 vial	2-8℃, 12 months, shading light
Preparation of 0.6mmol/L p-Nitrophenol standard working solution: dilute the reagent 5 with double distilled water for 5 times.			

Experimental instrument

Test tube, Centrifuge, Micropipettor, Vortex mixer, Water bath, Incubator, Spectrophotometer (400 nm)

Sample preparation

1. **Plasma (serum):** Detect directly.
2. **Urine:** Collect the fresh urine and centrifuge the sample at 10000 g for 10 min at 4℃. Take the supernatant for detection.

3. **1% Tissue homogenates:** Mince the tissues to small pieces, then be weighed and homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged at 2500 rpm for 10 min and collect the supernatant to prepare the 10% tissue homogenates. Then dilute the 10% tissue homogenates with normal saline for 10 times to prepare the 1% tissue homogenates. Meanwhile, determine the concentration of supernatant.

Operation steps

1. Serum (plasma) and urine sample

	Blank tube	Standard tube	Sample tube	Control tube
Double distilled water (mL)	0.1			
0.6mmol/L p-Nitrophenol standard working solution (mL)		0.1		
Sample (mL)			0.1	0.1
Reagent 1 (mL)	0.5	0.5		
Substrate solution (mL)			0.5	
Mix thoroughly and react at 37℃ for 15 min accurately.				
Reagent 3 (mL)	2	2	2	2
Substrate solution (mL)				0.5
Reagent 4 (mL)	0.05	0.05	0.05	0.05
Mix thoroughly. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 400 nm with 1 cm optical path cuvette.				

2. Tissue sample

	Blank tube	Standard tube	Sample tube	Control tube
Double distilled water (mL)	0.02			
3 mmol/L p-Nitrophenol standard stock solution (mL)		0.02		
Sample (mL)			0.02	0.02
Reagent 1 (mL)	0.5	0.5		
Substrate solution (mL)			0.5	
Mix thoroughly and react at 37℃ for 15 min accurately.				
Reagent 3 (mL)	2	2	2	2
Substrate solution (mL)				0.5
Reagent 4 (mL)	0.05	0.05	0.05	0.05
Mix thoroughly. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 400 nm with 1 cm optical path cuvette.				

Calculation of results

1. Serum (plasma) and urine sample

Definition: The amount of NAG in 1 L of sample react with substrate per minute at 37℃ that catalyze the production of 1 μmol of p-nitrophenol is defined as 1 unit.

$$\text{NAG activity (U/L)} = \frac{\text{OD}_{\text{Sample}}-\text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}}-\text{OD}_{\text{Blank}}} \times \text{Concentration of standard } (0.6\ mmol/L) \times \frac{1}{\text{Reaction time (15 min)}} \times 1000$$

Examples 1:

Take 0.1 mL of serum sample and carry the assay according to the operation table. The results are as follows: OD_{Blank} is 0.003, $OD_{Standard}$ is 0.436, $OD_{Control}$ is 0.148, and OD_{Sample} is 0.432, and the calculation result is:

$$\text{NAG activity (U/L)} = \frac{0.432-0.148}{0.436-0.003} \times 0.6 \times \frac{1}{15} \times 1000 = 26.236\ U/L$$

Examples 2:

Take 0.1 mL of urine sample and carry the assay according to the operation table. The results are as follows: OD_{Blank} is 0.003, $OD_{Standard}$ is 0.436, $OD_{Control}$ is 0.102, and OD_{Sample} is 0.140, and the calculation result is:

$$\text{NAG activity (U/L)} = \frac{0.140-0.102}{0.436-0.003} \times 0.6 \times \frac{1}{15} \times 1000 = 3.510\ U/L$$

2. Tissue sample

Definition: The amount of NAG in 1 g of tissue protein react with substrate per minute at 37℃ that catalyze the production of 1 μmol of p-nitrophenol is defined as 1 unit.

$$\text{NAG activity (U/gprot)} = \frac{\text{OD}_{\text{Sample}}-\text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}}-\text{OD}_{\text{Blank}}} \times \text{Concentration of standard } (3\ mmol/L) \times \frac{1}{\text{Reaction time (15 min)}} \times 1000 \div \text{Concentration of protein in 1\% tissue homogenates (gprot/L).}$$

Examples 3:

Take 0.02 mL of 1% rat renal cortical tissue homogenates sample and carry the assay according to the operation table. The results are as follows: OD_{Blank} is 0.003, $OD_{Standard}$ is 0.392, $OD_{Control}$ is 0.065, and OD_{Sample} is 0.519, the concentration of protein in sample is 1.241 gprot/L, and the calculation result is:

$$\text{NAG activity (U/gprot)} = \frac{0.519-0.065}{0.392-0.003} \times 3 \times \frac{1}{15} \times 1000 \div 1.241 = 188.09\ \text{U/gprot}$$

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. Since the concentration of the urease varies with the urinary flow rate, the measurement of NAG activity in urine requires 24 hours urine sample. It is quite inconvenient to keep the urine sample for 24 hours and this process may also affect the accuracy of the test. Thus, the ratio of “enzyme activity unit/creatinine” can be used to calculate the NAG activity in urine.