β-N-acetyl-glucosaminidase assay kit

(Cat/No.:BC120 Size:50T/24S)

1. Assay principle (Colorimetric Method)

N-acetyl-β-d-glucosaminidase (NAG) widely exists in various tissues, organs, body fluids, erythrocytes, leucocytes & blood platelets, etc, it is a acidic hydrolase in lysosomes. Under catalysis of NAG, substrate hydrolyzes and releases free p-nitrophenol. Alkaline solution is added to terminate reaction and induct p-nitrophenol coloration. It is able to calculate NAG activity by measuring OD values at 400nm.

2. Composition & preparation(6 months shelf life)

Reagent 1: 40ml×1 bottle;

Reagent 2: Substrate powder×1 vial;

Reagent 3: Terminator 60ml×2 bottles;

Reagent 4: Liquid 6ml×1 bottle (crystals will seed out in cold days, before use, place it in 37℃ water bath until it becomes limpid.)

Reagent 5: 3mmol/L p-nitrophenol standard stock solution 2m×1 bottle.

0.6mmol/L p-nitrophenol standard working solution preparation:
Dilute p-nitrophenol standard stock solution 5 times (V(3mmol/L p-nitrophenol):V(Double distilled water)=1:4)

Substrate preparation: This substrate has low solubility. When you prepare substrate solution, please mix sufficient quantum Reagent 1 with Reagent 2 at first (mixture appears pasty), then add Reagent 1 until volume reaches to 30ml (with stirring), mix sufficiently until dissolve completely (without heating). Prepared substrate buffer is supersaturated solution, if crystals seed out, then place

quescently or centrifugation and them take supernatant to use.
Residuary substrate buffer can be stored at 4℃ for more than 2 months.

3. Operation procedure

	Blank	Standard	Sample	Contrast
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 buffer (ml)			0.5	
Mix sufficiently, react at 37℃ for 15 minutes accurately				
Reagent 3 (ml)	2	2	2	2
Substrate buffer (ml)				0.5
Reagent 4 (ml)	0.05	0.05	0.05	0.05
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 400nm (adjust zero by distilled water).				

4. Calculation

（1）. Definition: 1 L sample reacts with substrate at 37℃ for 1 minute, 1μmol p-nitrophenol producing by hydrolysis is considered at 1 NAG activity unit (U).

（2）Formula:

$$\text{NAG activity (U/L)} = \frac{OD_{Sample} - OD_{Contrast}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (0.6mmol/L)} \times \frac{1}{15^*} \times 1000$$

* Reaction time is 15 minutes.

(3) Examples:

① Take 0.1ml blood serum to measure NAG activity, in results, OD_{Blank} is 0.003, $OD_{Standard}$ is 0.436, $OD_{Contrast}$ is 0.148, OD_{Sample} is 0.432, calculate as follows:

$$\text{NAG activity (U/L)} = \frac{OD_{Sample} - OD_{Contrast}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (0.6mmol/L)} \times \frac{1}{15} \times 1000$$

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

② Take 0.1ml urine to measure NAG activity, in results, OD_{Blank} is 0.003, $OD_{Standard}$ is 0.436, $OD_{Contrast}$ is 0.102, OD_{Sample} is 0.140, calculate as follows:

$$\text{NAG activity (U/L)} = \frac{OD_{Sample} - OD_{Contrast}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (0.6mmol/L)} \times \frac{1}{15} \times 1000$$

$$= \frac{0.140 - 0.102}{0.436 - 0.003} \times 0.6 \times \frac{1}{15} \times 1000 = 3.510 \quad U/L$$

5. Announcements

①. Linear range: OD≤0.60, NAG activity≤60 U/L. If sample is out of this range, then dilute sample with physiological saline and assay again, result should be multiplied with dilution times.

②. Urine NAG content alternates by urine flow rate, so it needs to retain all urine in 24 hours to

measure urine NAG. It is inconvenient and inaccurate to retain all urine in 24 hours, so it is better to reflect NAG discharging rate by "NAG unit/creatinine".

6. Points to Note

1. When using a spectrophotometer, the absorbance is linear up to 0.60 or the enzyme activity is linear up to 60 units. If the absorbance exceeds this range, the sample should be diluted with physiological saline and retested, and the result multiplied by the dilution factor.

2. Urinary enzyme concentration varies with urine flow rate; therefore, 24-hour urine collection is required for urinary enzyme testing. However, since collecting 24-hour urine is inconvenient and inaccurate, calculating the enzyme excretion rate using the "enzyme units/creatinine" ratio is more effective.

Appendix Ⅰ: NAG Assay in Rat cortex renis

1. Pretreatment:

Weigh tissue accurately, add 9 times physiological saline according to mass(g) -volume(ml) ratio of 1:9, make 10% tissue homogenate in icewater bath , centrifugate at 2500rpm for 10 minutes. Take supernatant, dilute with physiological saline to 1% tissue homogenate for assay

2. Operation Procedure:

	Blank	Standard	Sample	Contrast
Distilled water (ml)	0.02			
0.6mmol/L p-nitrophenol standard working solution (ml)		0.02		
Sample (ml)			0.02	0.02
Reagent 1 (ml)	0.5	0.5		
Substrate buffer (ml)			0.5	
Mix sufficiently, react at 37℃ for 15 minutes accurately				
Reagent 3 (ml)	2	2	2	2
Substrate buffer (ml)				0.5
Reagent 4 (ml)	0.05	0.05	0.05	0.05
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 400nm (adjust zero by distilled water).				

3. Calculation

(1) Definition: 1g tissue protein reacts with substrate at 37℃ for 1 minute, 1μmol p-nitrophenol producing by hydrolysis is considered at 1 NAG activity unit (U).

Formula:

$$\text{NAG activity (U/gprot)} = \frac{OD_{Sample} - OD_{Contrast}}{OD_{Satndard} - OD_{Blank}} \times \frac{\text{Standard concentration (3mmol/L)}}{\text{Sample Protein concentration (gprot/L)}} \times \frac{1000}{15} \div \text{Sample Protein}$$

* Reaction time is 15 minutes.

(2) Examples:

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Take 0.02ml 1% rat cortex renis homogenate to measure NAG activity, in results, OD_{Blank} is 0.003, $OD_{Standard}$ is 0.392, $OD_{Contrast}$ is 0.065, OD_{Sample} is 0.519, , protein concentration in 10% rat brain homogenate is 1.241g/L, calculate as follows:

$$\text{NAG activity (U/gprot)} = \frac{OD_{Sample} - OD_{Contrast}}{OD_{Satndard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \times \frac{1000}{15} \div \text{Sample Protein concentration (gprot/L)}$$

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