beta-N-Acetylglucosaminidase

Microplate Assay Kit

User Manual

Catalog # CAK1190

(Version 1.3C)

Detection and Quantification of beta-N-Acetylglucosaminidase (NAG) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

β-N-Acetylglucosaminidase (NAG, EC 3.2.1.52) is a lysosomal enzyme that is expressed in various tissues, including kidney, liver and lungs. NAG can cleave N-acetyl-glucosamine, a monosaccharide derivative of glucose. NAG concentration in urine is minimal due to its inability to cross the glomerular basal membrane. Increased concentration of NAG in urine indicates renal tubular cell breakdown. Acute Kidney Injury (AKI) is the sudden loss of kidney functions, causing electrolyte imbalance, and retention of urea and other nitrogenous products. NAG has become one of the most studied and used biomarkers for the detection and diagnosis of AKI. beta-N-Acetylglucosaminidase Microplate Assay Kit is a sensitive assay for determining beta-N-Acetylglucosaminidase activity in various samples. In this assay, NAG uses a synthetic p-nitrophenol derivative (R-pNP) as a NAG substrate and releases pNP which can be measured at absorbance. The intensity of the product color, measured at 405 nm, is proportional to the NAG activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	50 µl x 1	-20 °C
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Note:

Substrate: add 9 ml Reaction Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

Standard: add 1 ml distilled water to dissolve before use, mix; then add 30 µl into 970 µl distilled water, the concentration will be 300 µmol/L; store at -80 °C for a month after reconstitution. Perform 2-fold serial dilutions with distilled water.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Serum samples can be used directly. Centrifuge urine samples at 10,000 x g, 4°C for 3 mins, if precipitation is observed, collect supernatant.

V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Substrate	90 µl	90 µl	--	--	90 µl
Sample	10 µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Standard	--	--	100 µl	--	--
Positive Control	--	--	--	--	10 µl
Distilled water	--	--	--	100 µl	--
Mix, put it in the oven, incubate at 37 °C for 10 minutes.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, measured at 405 nm and record the absorbance.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of NAG activity is the amount of enzyme that generates 1 μmol of pNP per min at pH 4.2 at 37°C.

1. According to the protein concentration of sample

$$NAG\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$NAG\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$NAG\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 300 μmol/L = 0.3 μmol/ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

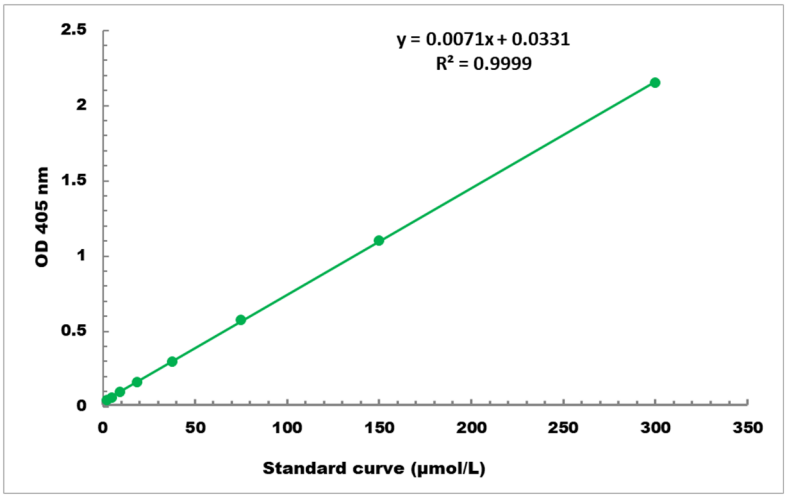
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml;

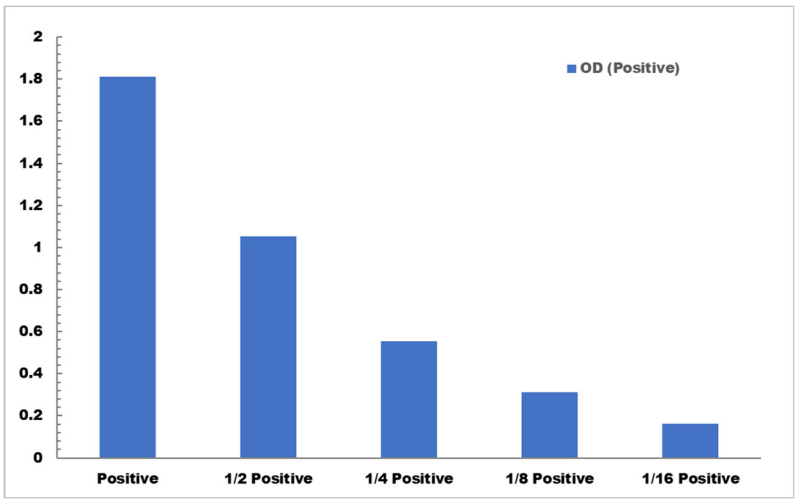
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 3 μmol/L - 300 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES