beta-N-Acetylglucosaminidase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1190

(Version 1.4C)

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

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-AcetylglucosaminidaseActivity Colorimetric 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 at405 nm, is proportional to the NAG activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 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 µlx 1	-20 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml Reaction Bufferto generate 10 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to 300 µmol/Lstandard top solutionby adding 30 µlstock solution into 970 µlReaction Buffer. Perform 2-fold serial dilutions of the top standardsolution using Reaction Bufferto make the standard curve. The concentration of standard curve could be 300/150/75/37.5/18.8/9.4/4.7µmol/L.

Substrate: Add 9 ml Reaction Buffer to dissolve before use; store at -80 °C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 mlAssay 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 liquidsamples

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.

VI. ASSAY PROCEDURE

Warm all reagents to 37°C before use.

Add following reagents intothe 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 into the oven, incubate at 37 °C for 10 minutes.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, measure at 405 nm and record the absorbance.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

2.4According to the quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times N \times (V_{Sample}/V_{Assay})\times T}(U/10^4)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

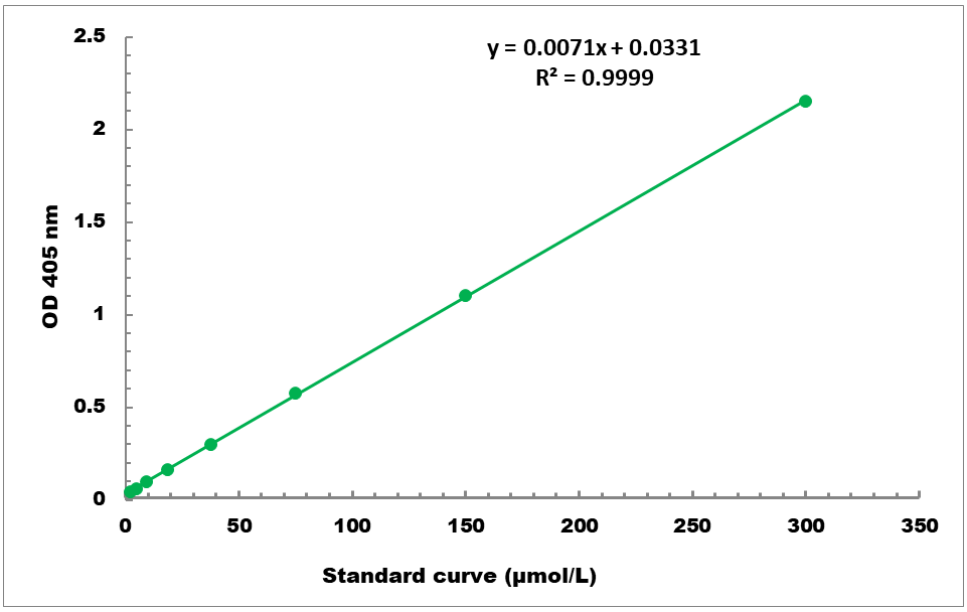
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

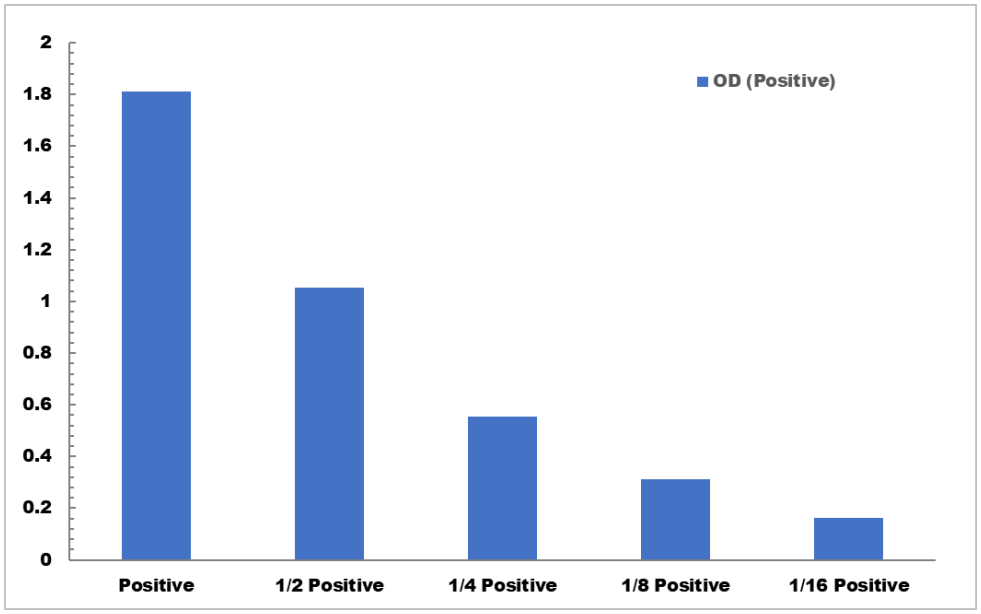
T: the reaction time, minute

VIII. 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