



# beta-N-Acetylglucosaminidase Microplate Assay Kit User Manual

Catalog # ASK1190

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

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

Bioworld Technology, Inc. (USA)

Email: [info@bioworld.com](mailto:info@bioworld.com)

Web: [www.bioworld.com](http://www.bioworld.com)

Bioworld technology, co. Ltd. (China)

Email: [info@biogot.com](mailto:info@biogot.com)

Web: [www.biogot.com](http://www.biogot.com)



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**I. INTRODUCTION**

beta-N-Acetylglucosaminidase (NAG) is a lysosomal enzyme involved in a variety of biological processes such as the degradation of glycoproteins and glycolipids, cell proliferation, and signal transduction. NAG is found in many tissues in the body, but due to its high molecular weight, it can not be filtered through the glomerular membrane. For this reason, in the presence of tubular damage or a glomerular lesion, urinary NAG activity increases. Elevated NAG levels in urine are an early indication of renal damage, such as injury due to diabetes mellitus, inflammation, nephritic syndrome, urinary tract infection, and more. Various forms of cancer have been associated with increased levels of NAG in serum. Genetically inherited lipid storage disorders, such as Tay-Sachs and Sandhoff disease, arise from deficiencies of the enzyme.

beta-N-Acetylglucosaminidase Microplate Assay Kit is based on the cleavage of p-nitrophenol from a synthetic substrate. p-Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.



**II. KIT COMPONENTS**

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Stop Solution	10 ml x 1	4 °C
Standard (500 µmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

**Note:**

Substrate: add 5 ml distilled water to dissolve before use.

**III. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. 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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 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**

For serum and plasma samples, detect directly.

For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.



**V. ASSAY PROCEDURE**

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 $\mu$ l	--	--
Substrate	50 $\mu$ l	--	--
Mix, put it in the oven, 37 °C for 20 minutes.			
Standard	--	100 $\mu$ l	--
Stop Solution	100 $\mu$ l	100 $\mu$ l	200 $\mu$ l
Mix, record absorbance measured at 405 nm.			

**VI. CALCULATION**

**Unit Definition:** One unit of NAG activity is defined as the enzyme generates 1  $\mu\text{mol}$  of p-nitrophenol per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{NAG (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (C_{\text{Protein}} \\ &\quad \times V_{\text{Sample}}) / T \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{NAG (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NAG (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times N / V_{\text{Assay}}) / T \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

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

$C_{\text{Standard}}$ : the concentration of Standard, 500  $\mu\text{mol/L}$  = 0.5  $\mu\text{mol/ml}$ ;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

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

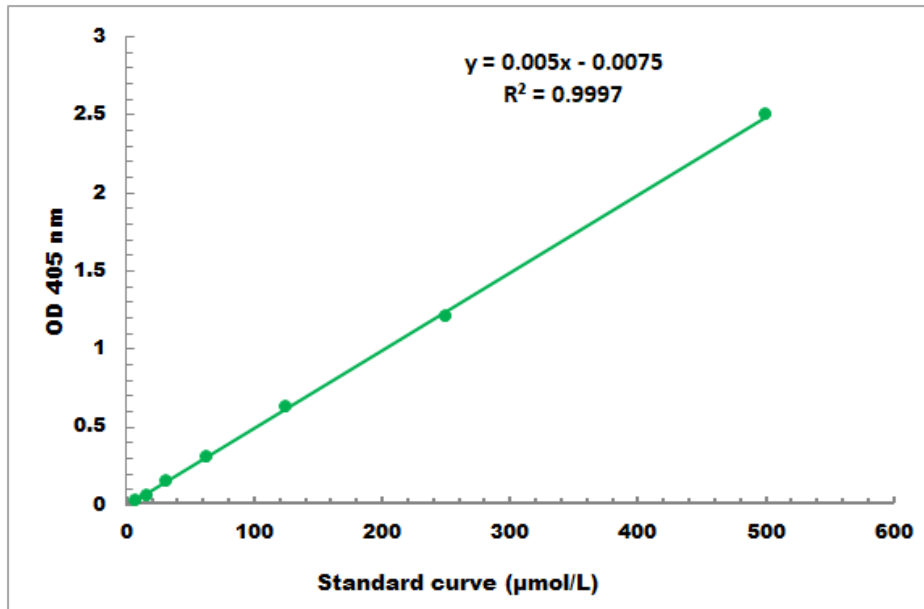
$V_{\text{Sample}}$ : the volume of sample, 0.05 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 20 minutes.

**VII. TYPICAL DATA**

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



Detection Range: 5 µmol/L - 500 µmol/L