beta-N-Acetylglucosaminidase

Microplate Assay Kit

Cat #: orb545640 (manual)

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.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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.

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.

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. Ice
7. Centrifuge
8. Timer

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

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.

ASSAY PROCEDURE

Add following reagents into the microplate:

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

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.

CALCULATION

Unit Definition: One unit of NAG activity is defined as the enzyme generates 1 μmol of p-nitrophenol per minute.

1. According to the protein concentration of sample

$$NAG\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$
$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$NAG\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W/ V_{Assay}) / T$$
$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$NAG\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N/ V_{Assay}) / T$$
$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of Standard, 500 μmol/L = 0.5 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

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

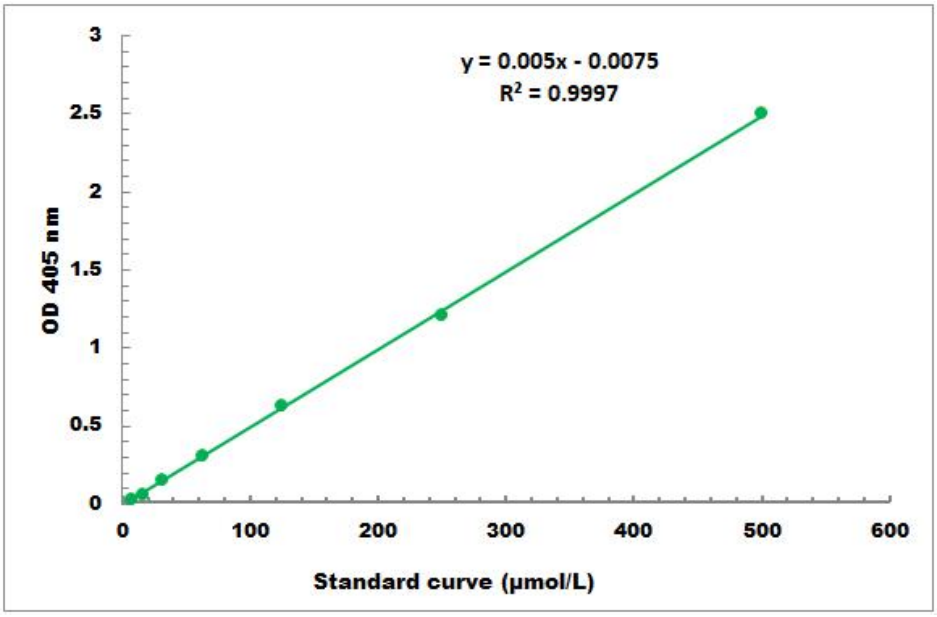
V_{Sample}: the volume of sample, 0.05 ml;

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

T: the reaction time, 20 minutes.

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