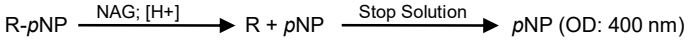
β-N-Acetylglucosaminidase Activity Assay Kit (Colorimetric) (#BN00952)

(Catalog #BN00952; 100 assays; Store at -20°C)

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. Its 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. Assay Genie's NAG Activity Assay Kit provides a simple and sensitive method for monitoring NAG enzymatic activity. In this assay, NAG uses a synthetic p-nitrophenol derivative (R-*p*NP) as a NAG substrate and releases *p*NP which can be measured at absorbance (OD 400 nm). The assay can detect as low as 50 µU of NAG activity in a variety of samples.



II. Application:

- Measurement of NAG in biological samples from different mammalian species

III. Sample Type:

- Biological fluids such as urine, serum etc.
- Tissues such as kidney, liver, brain etc.
- Cells such as HeLa, HepG2, CHO etc.

IV. Kit Contents:

Components	BN00952	Cap Code	Part Number
NAG Assay Buffer	35 ml	NM	BN00952-1
NAG Substrate	6 ml	Amber	BN00952-2
p-Nitrophenol (*p*NP) (20 mM)	0.1 ml	Yellow	BN00952-3
NAG Stop Solution	3 ml	Clear	BN00952-4
NAG Positive Control	1 vial	Blue	BN00952-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA plate reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **NAG Assay Buffer**: Bring to room temperature (RT) before use. Store at 4°C or -20°C.
- **NAG Substrate:** Light sensitive. Store at -20°C. Once opened, use within two months. Bring to RT before use. Mix well. If precipitation is observed, sonicate the contents in a water bath sonicator (interval: 2 min). Repeat if necessary.
- ***p*-Nitrophenol (*p*NP):** Light sensitive. Bring to RT before use. Store at -20°C. Once opened, use within two months.
- **NAG Stop Solution:** Bring to RT before use. Store at -20 °C. Once opened, use within two months.
- **NAG Positive Control:** Reconstitute with 40 µl NAG Assay Buffer. Mix well. Store at -20°C. Use within two months.

VII. NAG Activity Assay Protocol:

1. **Sample Preparation:** Serum samples can be used directly. Centrifuge urine samples at 10,000 x g, 4°C for 3 min., if precipitation is observed. Collect supernatant. Homogenize tissues (~10-20 mg) or cells (~1x10^6) on ice using 100-200 µl NAG Assay Buffer. Centrifuge homogenate at 10,000 x g, 4°C for 3 min. Collect supernatant. Add 1-70 µl sample into desired well(s) in a 96-well plate. Prepare a parallel sample well as sample background control. For Positive Control, add 1-5 µl of Positive Control into desired well(s). Adjust the volume of Positive Control, background control and sample wells to 70 µl/well with NAG Assay Buffer.

   Note:

   NAG enzymatic activity varies over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve linear range.

2. **Standard Curve Preparation:** Prepare 2 mM *p*NP Standard by adding 10 µl of 20 mM *p*NP Standard into 90 µl of NAG Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of 2 mM *p*NP Standard into a series of wells in a 96-well plate to generate 0, 4, 8, 12, 16 and 20 nmol of *p*NP/well respectively. Adjust the volume to 125 µl/well with NAG Assay Buffer.
3. **Substrate Hydrolysis:** Add 55 µl NAG Substrate into each well containing samples, and positive controls. To the sample background control well, add 55 µl NAG Assay Buffer. Mix well. Incubate for 5-30 min. at 37°C. After incubation, add 25 µl NAG Stop Solution into each well containing samples, Positive Control, background control and Standards. Mix well.

   Note:

   Incubation time depends on the NAG enzymatic activity in samples. Longer incubation time may be required for samples having low NAG activity.

4. **Measurement:** To stabilize the reaction, incubate plate at 37°C for 10 min., protected from light. Measure absorbance (OD 400 nm) with end point setting.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the *p*NP Standard Curve. If sample background control reading is significant, then subtract sample background control reading from sample reading. Apply sample corrected OD to *p*NP Standard Curve to get B nmol of *p*NP generated by NAG activity in the sample well.

Sample NAG Activity = (BXD)/(TXV) nmol/min/ml or mU/ml or U/L

Where: **B** is amount of *p*NP in the sample well from Standard Curve (nmol)
T is incubation time for substrate hydrolysis (5-30 min.)
V is sample volume added into the reaction well (ml)
D is sample dilution factor

Sample NAG activity can also be expressed as mU/mg of protein or mU/mg creatinine (urine).

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

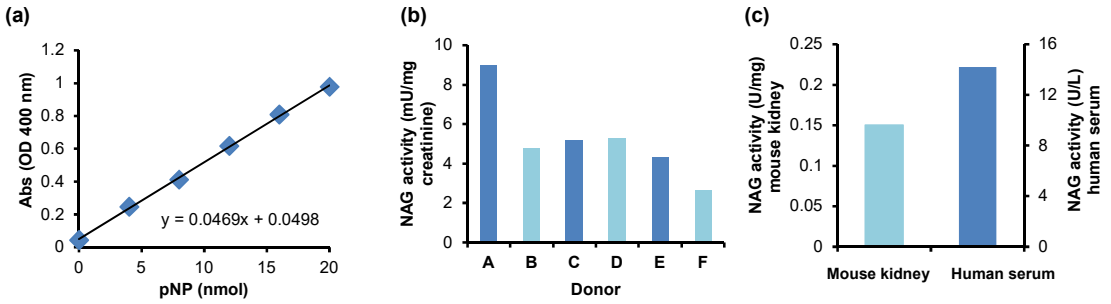


Figure: (a) *p*NP Standard Curve. (b) Measurement of NAG activity in human urine from different donors. Undiluted samples (70 µl) were incubated for 30 min. with NAG substrate. (c) Measurement of NAG activity in mouse kidney (10 µg) and human serum (20 µl). Samples were incubated for 30 min. with NAG substrate. All assays were performed following the kit protocol.

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