# GSI Beta-N-Acetylhexosaminidase Colormetric Assay Kit DataSheet

Beta-N-Acetylhexosaminidase (EC 3.2.1.52) also known as N-acetyl-beta-glucosaminidase (NAG), beta-hexosaminidase, hexosaminidase, OGA and OGT, is a lysosomal enzyme involved in the hydrolysis of terminal N-acetyl-D-hexosamine residues in N-acetyl-β-D-hexosaminides.[1][2] Functional β-hexosaminidases are dimers and three isozymes are produced through the combination of α and β subunits to form any one of three active dimers:[3] Gene mutations lead to hexosaminidase deficiency that causes numerous diseases such as Tay–Sachs disease.[4][5] Beta-hexosaminidase is expressed in various tissues and altered expression is involved in many diseases. OGA is upregulated in a wide range of human cancers and drives aerobic glycolysis and tumor growth by inhibiting pyruvate kinase M2 (PKM2).[6] PKM2 is dynamically O-GlcNAcylated in response to changes in glucose availability. Under high glucose conditions, PKM2 is a target of OGA-associated acetyltransferase activity, which facilitates O-GlcNAcylation of PKM2 by O-GlcNAc transferase (OGT). OGT promotes carcinogenesis and metastasis of cervical cancer cells. OGT's expression is significantly upregulated in cervical cancer, and low OGT level is correlated with improved prognosis.[7] Together with other tubular biomarkers such as kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin, NAG plays a vital role in patients with chest pain before contrast media exposition.[8]

**References**


1. Calvo P, et al. (1978). Biochem. J. 175 (2): 743–50. doi:10.1042/bj1750743.
2. Gao Y, et al. (2001) J. Biol. Chem. 276 (13), 9838-9845.
3. Hou Y, et al. (1996). Biochemistry. 35 (13): 3963–9. doi:10.1021/bi9524575.
4. Mark BL, et al. (2003). J. Mol. Biol. 327 (5): 1093–109.
5. Lemieux MJ, et al. (2006). J. Mol. Biol. 359 (4): 913–29. doi:10.1016/j.jmb.2006.04.004.
6. Singh JP, et al (2020) Oncogene 39 (3), 560-573.
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## PRINCIPLE OF THE ASSAY

Beta-N-Acetylhexosaminidase Colormetric Assay Kit provides a simple but sensitive method for monitoring hexosaminidase activity in biological samples (tissues, cells, plasma, serum, urine, etc). The assay uses a synthetic p-nitrophenol derivatives (R-pNP) as a substrate of hexosaminidase and release of pNP from the enzymatic reaction can be measured as optical density at wavelength of 405 nm.

## DECLARATION

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMAN AND ANIMALS.

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## Applications

This kit is for 100 tests to measure hexosaminidase activity in biological samples.

## Key Features

Fast and sensitive: linear detection range for 20 µL sample is 0.2 to 100 U/L for a 30 min reaction at 37 ºC.

High throughput: Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

This package insert must be read in its entirety before using this product.

## Storage

Store at -20 °C as supplied for up to 12 months.

MATERIALS PROVIDED

| Description | Quantity | Description | Quantity | Description | Quantity |
|---|---|---|---|---|---|
| Substrate,10 mL | 1 | Assay Buffer, 12 mL | 1 | Standard, 0.2 mL (10 mM nitrophenol) | 1 |
| Positive control, 50 µL | 1 | Stop Solution, 12 mL | 1 | DataSheet/Manual | 1 |

## Materials Not Provided But Required

96-well microplate, multi-channel pipettor, microplate reader

## Reagent Preparations

Equilibrate all components to 37 ºC and vortex briefly prior to use.

Preparation of Standard Stock (2 mM): prior to assay, prepare 2 mM Standard Stock by adding 50 µL of 10 mM nitrophenol (Standard) to 200 µL of Assay Buffer and mix thoroughly.

## Sample Preparations

Serum and plasma samples can be assayed directly. For urine samples containing precipitate, centrifuge at 10,000 x g, 4 ºC for 3 min and collect the supernatant for assay.

Cell lysate: collect cells by centrifugation at 2000 x g for 5 min at 4 ºC. For adherent cells, do not harvest cells using proteolytic enzymes, instead use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS approximately one million cells per milliliter. Centrifuge at 14,000 x g for 10 min at 4 ºC and collect supernatant for assay.

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop solutions to samples should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.

**Assay Procedure**

1. Standard preparation: prepare each of the standards as following:

| Standard | pNP (µM) | Standard stock, 2 mM (µL) | Assay Buffer (µL) | Volume (µL) |
|---|---|---|---|---|
| 1 | 2000 | 100 | 0 | 100 |
| 2 | 1000 | 50 | 50 | 100 |
| 3 | 500 | 25 | 75 | 100 |
| 4 | 250 | 12.5 | 87.5 | 100 |
| 5 | 125 | 6.25 | 93.75 | 100 |
| 6 | 62.5 | 3.125 | 96.875 | 100 |
| Blank | 0 | 0 | 100 | 100 |

2. Add 20 µl of sample, standard, positive control and blank to each well of 96-well plate.
3. For all samples, positive control and blank, add 80 µl of Substrate Solution to each well. For standards, add 80 µl of Assay Buffer to each Standard well (Attention: DO NOT add Substrate Solution to the Standard well). Tap plate briefly to mix thoroughly.
4. Incubate at 37 ºC or desired temperature for 30-60 min.
5. Add 100 µL of Stop Solution to all wells. Tap plate briefly to mix thoroughly.
6. Read optical density at 405 nm.

**Activity Calculation**

Subtract blank OD from the standard OD values and plot the ΔOD against standard concentration. Determine the slop and use the following equation to calculate hexosaminidase activity.

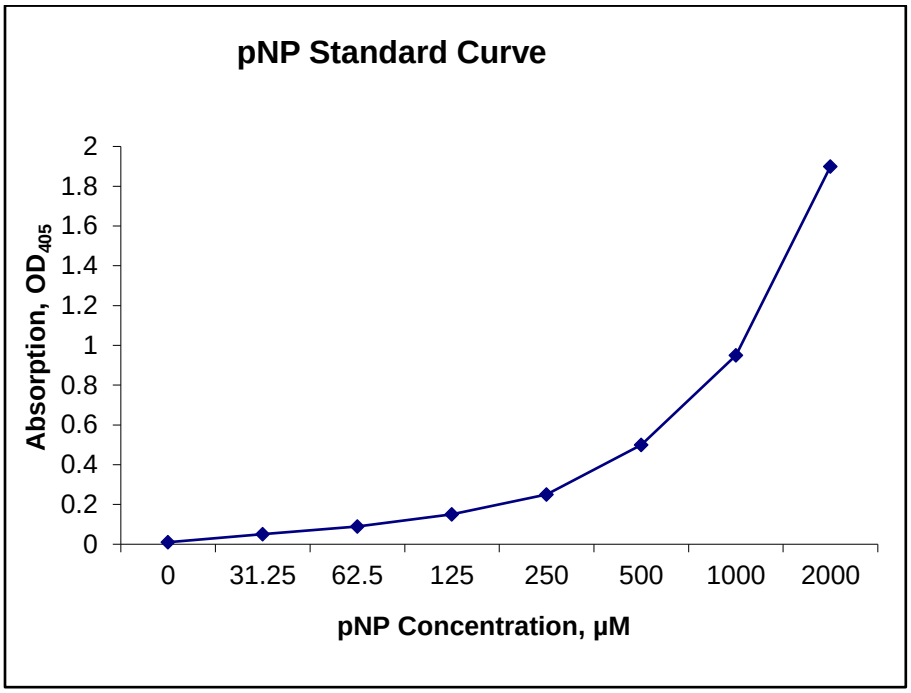
NGA Activity (U/L) = DF * ($OD_{sample}$ - $OD_{blank}$)/(t * Slope)

Where $OD_{sample}$ is the $OD_{405}$ value for each sample and $OD_{blank}$ is the $OD_{405}$ value for blank. Slop represents the linear regression fit of the standard points and t is the reaction time (min). DF is the dilution factor.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 µmole of pNitrophenol N-acetyl-D glucosamine per min at 37 ºC and pH 4.5.
Note: if sample NAG activity exceeds 200 U/L, either use a shorter reaction time or dilute sample in water and repeat the assay. For sample NGA activity < 1 U/L, the incubation time can be extended up to 4 h for greater sensitivity.

**Typical Data**

The standard curve is provided for demonstration only as shown below. A standard curve should be generated for each set of samples assayed.




**Precaution and Technical Notes**

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A negative control without addition of substrate is recommended and the $OD_{405}$ subtraction should be applied to each test to correct the background signal.
3. This kit should not be used beyond the expiration date on the label.
4. Use a fresh reagent reservoir and pipette tips for each step.
5. It is recommended that all controls and samples be assayed in duplicate.
6. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.