Catalog #80219

V.1a

Crystal Chem

N-Acetyl-β-D-Glucosaminidase (NAG) Kit Instructions

For the quantitative determination of NAG in urine

Catalog #80219
100 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The NAG Kit is for the quantitative determination of NAG in urine. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

NAG is a lysosomal enzyme involved in the breakdown metabolism of glycoproteins. Increased NAG levels in urine are an early indication of renal disease and can serve as a valuable renal monitoring test for various disorders.

C. Principle of the Assay

Crystal Chem's NAG Assay is based on the following principle: NAG hydrolyses 2-methoxy-4-(2'nitrovinyl)-phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (MNP-GlcNAc) to 2-methoxy-4-(2'-nitrovinyl)-phenol product. The product formation is detected by development of color at 505 nm upon addition of an alkaline buffer.

D. Kit Storage

1. Upon receipt of the NAG Kit, reagents should be stored at 2-8°C (do not freeze the kit or hold it at temperatures above 25°C). Calibrators should be stored separately and frozen at -20°C.
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (liquid)	1 X 15 mL
CC2	Reagent CC2 (liquid)	1 X 3 mL
CC3	Reagent CC3 (liquid)	1 X 6 mL
CAL1	Calibrator 1 (lyophilized)	1 X 2 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Clean glass tubes and test tube racks
Incubator (37ºC)
Deionized water
Spectrophotometer (should read A_{505} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (10 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the glass tubes from drying out and to get the best results, samples and reagents should be dispensed quickly into the tubes.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.
6. Reagents are light sensitive. Store in a dark place.

H. Sample Collection

Fresh urine samples should be used when possible. However, urine samples can be stored for one week at 2-8°C or up to 1 month at -20°C without significantly affecting NAG activity. Samples containing low amounts of preservative can be used (less than 0.02% sodium azide). NAG activity is pH sensitive; hence, urine samples should have a pH range between 4.0 and 8.0.

I. Assay Procedure

I.1. Preparation of reagents

Prior to running the assay, a solution mix of Reagent CC1 and Reagent CC2 must be prepared. Reagent CC1 and Reagent CC2 should be mixed in a volume ratio of 5:1 (5 volumes of CC1 and 1 volume of CC2) to make the CC1 + CC2 solution mix. Each test requires 150 µL of the solution mix. Reagent CC3 is provided as ready-to-use. All reagents should be brought to room temperature for at least 30 minutes prior to use. Before use, mix the reagents thoroughly by gentle agitation or swirling. Reagents should be stored at 2-8°C immediately after use.

Note: *The CC1 + CC2 solution mix, thus prepared, is stable for 1 week when stored capped at 2-8°C. Reagent CC3, once opened, is stable for 1 month at 2-8°C if closed tightly after use.*

I.2. Preparation of samples, calibrators, and controls

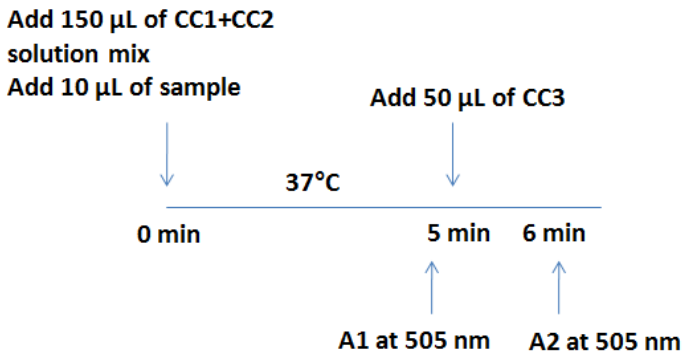
1. Prior to first use, calibrators and controls must be reconstituted with 2 mL of deionized water. Upon reconstituting, mix the reagents via gentle agitation or swirling. Reconstituted calibrator and controls should be equilibrated at 2-8°C for 24 hours prior to first use.
   Note: *Calibrators and controls are stable for two weeks at 2-8°C once reconstituted. Controls are sold separately (Cat# 80213).*
2. Prior to running the assay, bring all samples, calibrators, and controls to room temperature.
   Note: *In addition to running the calibrator provided, the assay requires running a blank calibrator. Deionized water should be used for running the blank calibrator.*

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers. Please contact Crystal Chem for more information.

1. Add 150 µL of Reagent CC1 + Reagent CC2 solution mix and 10 µL of sample, calibrator, or control into a clean glass tube and mix well by repeated pipetting.
2. Place glass tube in incubator (37ºC) and allow contents to equilibrate to 37ºC over 5 minutes.
3. Measure absorbance using a spectrophotometer (measure A_{505} values).
   Note: The NAG assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC3.
4. Pipette 50 µL of Reagent CC3 into the glass tube and mix well by repeated pipetting.
5. Measure the increase in absorbance using a spectrophotometer (measure A_{505} values) 1 minute after the addition of Reagent CC3.

Figure 1. Summary of assay procedure



I.4. Determining the NAG concentration

1. Calculate the change in absorbance ΔA (1 min ~ 0 min)

   $\Delta A = (OD_{505nm,\ 1\ min}) - (OD_{505nm,\ 0\ min})$

2. Using linear graph paper, construct the NAG calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding lithium concentration on the X axis.
   Note: *Calibrator values vary per lot and should be obtained from the calibrator labels. A calibration curve should be plotted every time the assay is performed.*
3. NAG concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The NAG concentration is expressed as IU/L. This interpolation can be simplified using Equation 1 below.
   Note: *Samples with a high NAG concentration (200 IU/L or higher) should be diluted with deionized water (1:2 or 1:5) and rerun.*

   Equation 1. Calculation of NAG concentration

   NAG concentration =
   [(sample A_{505} – blank A_{505}) / (calibrator A_{505} – blank A_{505})] × calibrator conc.

J. Performance characteristics

J.1. Assay range

The NAG assay has a linear range from 0 - 200 IU/L.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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