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

N-Acetyl-β-D-Glucosaminidase (NAG)

Version: 20
Content Version: November 2021

Colorimetric assay for the determination of N-Acetyl-β-D-Glucosaminidase in urine.

Cat. No. 10 875 406 001 1 test combination
50 tests

Store the product at +15 to +25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	N-Acetyl-β-D-Glucosaminidase (NAG), Buffer Solution	Contains citric acid and potassium citrate.	1 bottle, 860 mg
2	N-Acetyl-β-D-Glucosaminidase (NAG), Substrate Solution	▪ Lyophilized ▪ Contains sodium 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide and borax.	1 bottle, 10 ml
3	N-Acetyl-β-D-Glucosaminidase (NAG), Stop Reagent	Contains sodium carbonate.	1 bottle, 3.5 g

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Buffer Solution	Store at +15 to +25°C.
2	Substrate Solution	
3	Stop Reagent	

1.3. Additional Equipment and Reagent required

For preparation of working solutions

- Double-distilled water

1.4. Application

The N-Acetyl-β-D-Glucosaminidase (NAG) test is intended for research studies. It is used to explore the assumed relationship between physiological disturbances and the appearance of NAG in urine.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use urine as sample material.

⚠ ***The activity determination of the N-acetyl-β-D-glucosaminidase (NAG) should be carried out directly after collecting the sample. Turbid urines should be centrifuged and the supernatant decanted. NAG is stable for one week at +2 to +8°C and for one month when stored at −15 to −25°C.***

Control Reactions

Use a reagent blank for the NAG assay since false-positive values may be found.

- These values are on average between 4 and 6 U/l too high.
- The reagent blank rises from 4.5 to 6 U/l within 5 hours and should therefore be reassayed for each series of measurements.

Working Solution

Solution	Preparation/Composition	Storage and Stability
Buffer Solution	Dissolve the contents of Bottle 1 with 55 ml double-distilled water.	–
Substrate Solution	Dissolve the contents of Bottle 2 with 55 ml of Buffer Solution.	Store 1 month at +2 to +8°C. ⚠ ***Keep protected from light.***
Stop Reagent	Dissolve the contents of Bottle 3 with 110 ml double-distilled water.	Store 1 month at +2 to +8°C.

2.2. Protocols

N-Acetyl-β-D-Glucosaminidase (NAG) assay

See section, **Working Solution** *for additional information on preparing solutions.*
Set up the assay using the following parameters:

Parameter	Value
Wavelength	580 nm
Light path	1 cm
Measuring volume	3.05 ml
Incubation temperature	+37°C

Measurement against reagent blank. One reagent blank is sufficient for each series of measurements.

1. Pipette the following solution into test tubes:

Solution	Sample [ml]	Reagent Blank [ml]
Substrate Solution	1.00	1.00

2. Incubate for 5 minutes at +37°C.

3. Add sample as shown in the table:

Sample type	Sample [ml]	Reagent Blank [ml]
Urine	0.05	–[1]

4. Mix and incubate for exactly 15 minutes at +37°C; use a stop watch.

5. Add Stop Reagent as shown in the table:

Reagent	Sample [ml]	Reagent Blank [ml]
Stop Reagent	2.00	2.00

6. Mix and allow to stand for 10 minutes at +37°C.
   – Measure at +15 to +25°C against the reagent blank within 50 minutes = A_{sample} for 15 minutes.
   If the NAG activity in the sample is too low, increase the incubation time to 30 or 60 minutes. The longer incubation time must then be taken into account in the calculation.

[1] 0.05 ml water can be omitted.

Calculation

$$\frac{1000 \times V}{40.67 \times 1 \times v \times t} \times A\ (sample)$$

V: Total volume (ml)
v: Volume of urine sample (ml)
t: Incubation time (minutes)
A: Absorbance measured at 580 nm

Example

3-Cresolfulfonphthalein:
$E_{580\ nm}$ = 40.67 [l × $mmol^{-1}$ × cm^{-1}]

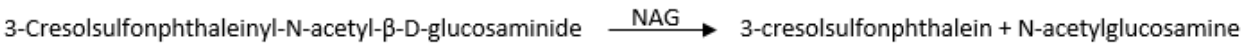
Volume activity

$$\frac{1000 \times 3.05}{40.67 \times 1 \times 0.05 \times 15} \times A\ (sample) = 100 \times A\ (sample)\ [U/l]$$

3. Additional Information on this Product

3.1. Test Principle

3-Cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide, sodium salt, is hydrolyzed by N-acetyl-β-D-glucosaminidase (NAG) with the release of 3-cresolsulfonphthalein, sodium salt (3-cresol purple), which is measured photometrically at 580 nm.



4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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