N-Acetyl-\(\beta\)-D-Glucosaminidase (NAG)

Colorimetric assay for the determination of N-Acetyl-\(\beta\)-D-Glucosaminidase in urine

Cat. No. 10 875 406 001
Test-Combination for approx. 50 tests

Store at +15 to +25°C

1. What this Product Does

Kit Contents

<table>
<thead>
<tr>
<th>Vial</th>
<th>Content</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer Substance</td>
<td>consisting of citric acid and potassium citrate.</td>
</tr>
<tr>
<td>2</td>
<td>Lyophilized Substrate</td>
<td>consisting of sodium 3-cresolsulphonphthaleinyl-N-acetyl-(\beta)-D-glucosaminide and borax.</td>
</tr>
<tr>
<td>3</td>
<td>Stop Reagent</td>
<td>consisting of sodium carbonate.</td>
</tr>
</tbody>
</table>

Storage and Stability
Stable when stored at +15 to +25°C until the expiration date printed on the labels.

Application
The 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

Preparation of Solutions
- **Buffer solution**: Dissolve the contents of bottle 1 with 55 ml double dist. water.
- **Substrate solution (solution II)**: Dissolve the contents of bottle 2 with 55 ml solution I.
- **Stop reagent (solution III)**: Dissolve the contents of bottle 3 with 110 ml double dist. water.

Stability of Solutions
Solution II is stable for 1 month when stored at +2 to +8°C, protected from light.
Solution III is stable for 1 month stored at +2 to +8°C.

Stability of the sample
The activity determination of the N-acetyl-\(\beta\)-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 (1).

Control Reaction
The inclusion of a reagent blank is necessary for the NAG assay, as otherwise false positive values may be found. Such 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 h and should therefore be reassayed for each series of measurements.

2.2 Procedure

<table>
<thead>
<tr>
<th>Pipette into test tubes</th>
<th>sample</th>
<th>reagent blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate solution (II)</td>
<td>1.00 ml</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>urine sample</td>
<td>0.05 ml</td>
<td></td>
</tr>
</tbody>
</table>

Mix and incubate for exactly 15 min at 37°C (stop watch).

Add

| stop reagent (III) | 2.00 ml | 2.00 ml |
| mix, allow to stand for 10 min at 37°C and measure at +15 to +25°C against the reagent blank within 50 min = \(A_{\text{sample}}\) for 15 min. |

\(0.05\) ml water can be neglected.

If the NAG activity in the sample is too low, then the incubation time can be increased to 30 or 60 min. The longer incubation time must then be taken into account in the calculation.

Calculation

\[
\frac{1000 \times V}{40.67 \times 1 \times v \times t} \times A\ (\text{sample})
\]

\(V\): total volume measured (in ml)
\(v\): volume of urine sample (in ml)
\(t\): incubation time (in min)
\(A\): absorbance measured at 580 nm

Example
3-Cresolsulfonphthalein:
\(E_{580\ \text{nm}} = 40.67\ \text{[}l \times \text{mmol}^{-1} \times \text{cm}^{-1}\]\)

Volume activity

\[
\frac{1000 \times 3.05}{40.67 \times 1 \times 0.05 \times 15} \times A\ (\text{sample}) = 100 \times A\ (\text{sample})\ [\text{U/l}]
\]
3. Additional Information on this Product

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

\[
\text{NAG} \rightarrow 3\text{-cresolsulfonphthalein} + \text{N-acetyl-glucosamine}
\]

Reference

4. Supplementary Information

4.1 Changes to Previous Version
- Editorial Changes

4.2 Ordering Information
For your further information: Roche Diagnostics offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage www.lifescience.roche.com

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack size</th>
<th>Cat. Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl-β-D-Glucosaminidase (NAG)</td>
<td>for approx. 50 tests</td>
<td>10 875 406 001</td>
</tr>
<tr>
<td>N-Acetyl-β-D-Glucosaminidase Control (NAG Control)</td>
<td>3 bottles</td>
<td>11 164 368 001</td>
</tr>
<tr>
<td>N-Acetyl-β-D-Glucosaminidase Standard (NAG Standard)</td>
<td>Lyophilizate for 5 × 3 ml</td>
<td>10 982 962 001</td>
</tr>
</tbody>
</table>

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