Enzymatic Assay of β-N-ACETYLGUCOSAMINIDASE
(EC 3.2.1.30)

PRINCIPLE:

PNP-NAG + H₂O → p-Nitrophenol + NAG

Abbreviations:
PNP-NAG = p-Nitrophenyl N-Acetyl-β-D-Glucosaminide
NAG = N-Acetyl-β-D-Glucosamine

CONDITIONS: T = 37°C, pH = 4.8, A₄₀₅nm, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Citrate Buffer
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate.)

B. 200 mM Sodium Phosphate Buffer
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous.)

C. Citrate Phosphate Buffer, pH 4.8 at 37°C (Cit-Phos)
(Prepare by adjusting the pH of Reagent A with Reagent B.)

D. 2.0 mM p-Nitrophenyl N-Acetyl-β-D-Glucosaminide Solution with 0.1% (w/v) Bovine Serum Albumin (PNP-NAG)
(Prepare 10 ml in Reagent C using p-Nitrophenyl N-Acetyl-β-D-Glucosaminide, and Albumin, Bovine.)

E. 1 M Sodium Carbonate Solution (Na₂CO₃)
(Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous.)

F. β-N-Acetylglucosaminidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of β-N-Acetylglucosaminidase in cold Reagent C.)
Enzymatic Assay of β-N-Acetylglucosaminidase (EC 3.2.1.30)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Cit-Phos)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent D (PNP-NAG)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 37°C. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate for exactly 5 minutes at 37°C. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Na₂CO₃)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>----</td>
</tr>
</tbody>
</table>

Mix by swirling and transfer to suitable cuvettes. Record the A₄₀⁵nm for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A_{405\text{nm}} \text{ Test} - A_{405\text{nm}} \text{ Blank}) \times (3.1) \times (\text{df})}{(5) \times (18.5) \times (0.1)}
\]

3.1 = Total volume (in milliliters) of assay
5 = Time of assay (in minutes)
18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405nm
0.1 = Volume (in milliliter) of enzyme used

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl N-acetyl-β-D-glucosaminide to p-nitrophenol and N-acetyl-β-D-glucosamine per minute at pH 4.8 at 37°C.
Enzymatic Assay of β-N-ACETYLGLUCOSAMINIDASE
(EC 3.2.1.30)

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 0.91 mM p-nitrophenyl N-acetyl-β-D-glucosaminide, 0.05% (w/v) bovine serum albumin and 0.002 – 0.004 unit of β-N-acetylglucosaminidase. The final concentrations of citric acid and sodium phosphate varies.

REFERENCES:


NOTES:

1. The extinction coefficient is described in Bowers, Jr., G.N. et al. (1980).

2. This assay is based on the cited references.

3. Where OUR Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.