Enzymatic Assay of α-N-ACETYLGALACTOSAMINIDASE
(EC 3.2.1.49)

**PRINCIPLE:**
PNP-NAG + H₂O → α-N-Acetylgalactosaminidase → NAG + p-Nitrophenol

Abbreviation used:
PNP-NAG = p-Nitrophenyl N-Acetyl-α-D-Galactosaminide

**CONDITIONS:**  T = 37°, pH = 3.65, A₄₀₀nm, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

A. 100 mM Citrate and 100 mM Sodium Phosphate Buffer, pH 3.65 at 37°C
   (Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, and Sodium Phosphate, Monobasic, Monohydrate. Adjust to pH 3.65 at 37°C with either 1 M NaOH or 1 M HCl.)

B. 100 mM Citrate and 100 mM Sodium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 3.65 at 37°C (Enzyme Diluent)
   (Prepare 25 ml in Reagent A using Albumin, Bovine.)

C. 5.0 mM p-Nitrophenyl N-Acetyl-α-D-Galactosaminide (PNP-NAG)
   (Prepare 2 ml in Reagent A using p-Nitrophenyl N-Acetyl-α-D-Galactosaminide.)

D. 200 mM Borate Buffer, pH 9.8 at 25°C (Borate)
   (Prepare 25 ml in deionized water using Boric Acid. Adjust to pH 9.8 at 25°C with 1 M NaOH.)

E. α-N-Acetylgalactosaminidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.02 – 0.05 unit/ml of α-N-Acetylgalactosaminidase in cold Reagent B.)
Enzymatic Assay of α-N-ACETYLGLUCOSAMINIDASE  
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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 (PNP-NAG)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.05</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Borate)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>------</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix by inversion and transfer to suitable cuvettes. Record the $A_{400\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

Units/ml enzyme = \[
\frac{(A_{400\text{nm}} \text{ Test} - A_{400\text{nm}} \text{ Blank}) \times (1.15) \times (df)}{(18) \times (10) \times (0.05)}
\]

1.15 = Total volume (in milliliters) of assay
df = Dilution factor
18 = Millimolar extinction coefficient$^1$ of p-Nitrophenol at 400 nm
10 = Time (in minutes) of assay per the Unit Definition
0.05 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will release 1.0 µmole of p-nitrophenol from p-nitrophenyl-N-acetyl-α-D-galactosaminide per minute at pH 3.65 at 37°C.
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FINAL ASSAY CONDITION:

In a 0.15 ml reaction mix, the final concentrations are 100 mM citric acid, 100 mM sodium phosphate, 0.03% (w/v) bovine serum albumin, 3.3 mM p-nitrophenyl N-acetyl-α-D-galactosaminide, and 0.001 - 0.0025 unit α-N-acetylgalactosaminidase.

REFERENCE:


NOTES:

1. The millimolar extinction coefficient of p-nitrophenol at 400 nm is described in Bessey, O.A., Lowry, O.H., and Brock, M.J. (1946).

2. This assay is based on the assay procedure described in Uda, Y., Li, S.-C., and Li, Y.-T. (1977).

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.