Enzymatic Assay of α-GALACTOSIDASE
(EC 3.2.1.22)

PRINCIPLE:

PNP α-D-Galactopyranoside + H₂O → α-Galactosidase → p-Nitrophenol + D-Galactose

Abbreviation used:
PNP α-D-Galactopyranoside = p-Nitrophenyl α-D-Galactopyranoside

CONDITIONS:  T = 25°C, pH = 6.5, A₄₀₅nm, Light path = 1 cm

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Monobasic Solution
   (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous.)

B. 100 mM Potassium Phosphate Dibasic Solution
   (Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate.)

C. 100 mM Potassium Phosphate Buffer, pH 6.5 at 25°C
   (Prepare 100 ml by adjusting 50 ml of Reagent A to pH 6.5 at 25°C by adding Reagent B.)

D. 9.9 mM p-Nitrophenyl α-D-Galactopyranoside Solution
   (PNP-Gal)
   (Prepare 4 ml in deionized water using p-Nitrophenyl α-D-Galactopyranoside.)

E. 200 mM Borate Buffer, pH 9.8 at 25°C
   (Prepare 100 ml in deionized water using Boric Acid, Adjust to pH 9.8 at 25°C with 1 M NaOH.)

F. α-Galactosidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.05 – 0.10 units/ml of α-Galactosidase in cold Reagent C.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
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<tbody>
<tr>
<td>Reagent C (Potassium Phosphate Buffer)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Reagent D (PNP-Gal)</td>
<td>0.20</td>
<td>0.20</td>
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</tbody>
</table>

Mix by swirling and equilibrate to 25°C. Then add:

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

Immediately mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

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<tbody>
<tr>
<td>Reagent E (Borate Buffer)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and record the A_{405nm} for both the Test and Blank, using a suitably thermostatted spectrophotometer.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A_{405nm \text{ Test}} - A_{405nm \text{ Blank}})(3.0)(df)}{(18.5)(5)(0.1)}
\]

3.0 = Total volume of assay  
df = Dilution factor  
5 = Conversion factor for 5 minutes to 1 minute  
18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm  
0.1 = Volume (in milliliter) of enzyme used

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

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

One unit will hydrolyze 1.0 µmole of p-nitrophenyl
α-D-galactoside to p-nitrophenol and D-galactose per minute
at pH 6.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are
80 mM potassium phosphate, 2.0 mM p-nitrophenyl
α-D-galctopyranoside, and 0.005 - 0.01 units α-
galactosidase.

REFERENCES:

(1968) Eur. J. Biochem. 8, 395

NOTES:

1. This assay is based on the cited reference.