Enzymatic Assay of α-GALACTOSIDASE  
(EC 3.2.1.22)

**PRINCIPLE:**

PNP α-D-Galactopyranoside + H₂O  \( \xrightarrow{\text{α-Galactosidase}} \)  p-Nitrophenol + D-Galactose

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

**CONDITIONS:**  \( T = 25^\circ C, \ pH = 6.5, \ A_{405nm}, \ \text{Light path} = 1 \ cm \)

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

A. 100 mM Potassium Phosphate Monobasic Solution  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

B. 100 mM Potassium Phosphate Dibasic Solution  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)

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, Sigma Prod. No. N-0877.)

E. 200 mM Borate Buffer, pH 9.8 at 25°C  
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. 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.)
Enzymatic Assay of α-GALACTOSIDASE  
(EC 3.2.1.22)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

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

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

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

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>-----</th>
</tr>
</thead>
<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$_{405\text{nm}}$ for both the Test and Blank, using a suitably thermostatted spectrophotometer.

CALCULATIONS:

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

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

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

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]
Enzymatic Assay of α-GALACTOSIDASE  
(EC 3.2.1.22)

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.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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