

**Enzymatic Assay of α -GALACTOSIDASE
(EC 3.2.1.22)**

PRINCIPLE:

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

Abbreviation used:

PNP α -D-Galactopyranoside = p-Nitrophenyl α -D-Galactopyranoside

CONDITIONS: T = 25°C, pH = 6.5, A_{405nm}, 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.)

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PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|--|-------------|--------------|
| Reagent C (Potassium Phosphate Buffer) | 0.70 | 0.70 |
| Reagent D (PNP-Gal) | 0.20 | 0.20 |

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

| | | |
|-----------------------------|------|-------|
| Reagent F (Enzyme Solution) | 0.10 | ----- |
|-----------------------------|------|-------|

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

| | | |
|-----------------------------|-------|------|
| Reagent E (Borate Buffer) | 2.00 | 2.00 |
| Reagent F (Enzyme Solution) | ----- | 0.10 |

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

CALCULATIONS:

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

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 μ mole 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.