

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.)
- 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, 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.)

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	-----
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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

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

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

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 -galactosida.