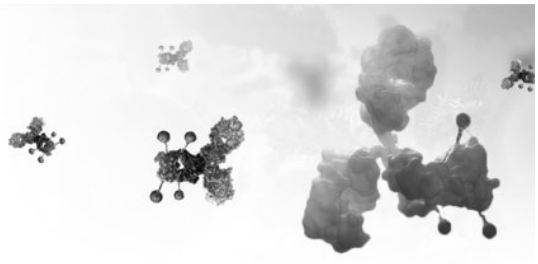

Enzymatic Assay of α-GALACTOSIDASE (CDLS-1)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Size 5 Unit

Principles of Testing

PNP α-D-Galactopyranoside + H_2O —α-Galactosidase→ p-Nitrophenol + D-Galactose

Abbreviation used:

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

Reagents And Materials Provided

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.)

Assay Procedure

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

	Test	Blank
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 A405nm		
for both the Test and Blank, using a suitably thermostatted spectrophotometer.		

Calculation

$$\text{Units/ml enzyme} = \frac{(\text{A405nm Test} - \text{A405nm Blank})(3.0)(df)}{(18.5)(5)(0.1)}$$

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

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

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

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

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