ADVANCED ENZYME TECHNOLOGIES LTD.

A-61/62, MALEGAON MIDC, SINNAR, NASHIK- 422113 (INDIA)

STANDARD ANALYTICAL PROCEDURE

TITLE : α-GALACTOSIDASE METHOD

Sr. No	Test	Method
01	Principle :	This procedure is used to determine α-galactosidase activity in enzyme preparations derived from *Aspergillus niger* var. The assay is based on a 15-min hydrolysis of p-nitrophenyl-α-D-galactopyranoside followed by spectrophotometric measurement of the liberated p-nitrophenol. One galactosidase activity unit (Gal U) is defined as the quantity of the enzyme that will liberate p-nitrophenol at the rate of 1 µmol/min under the conditions of the assay.
02	Reagent :	1. **Acetate Buffer.** Dissolve 11.55 ml of glacial acetic acid (CH_3COOH) in water, and dilute to 1000 ml (Solution A*)*. Dissolve 16.4 *g* of sodium acetate (CH_3COONa) in water, and dilute to 1000 ml (Solution B). Mix 7.5 ml of Solution A and 42.5 ml of Solution B, and dilute to 200 ml. Adjust the pH of this solution to 5.5 with either Solution A or B as necessary. **OR** (Dissolve 3.49 g of sodium acetate in 800 ml water and add 0.4 ml of acetic acid, adjust the pH to 5.5 and dilute 1000 ml.) 2. **Substrate Solution.** Dissolve 0.105 g of p-nitrophenyl-α-D-galactospyranoside (Sigma Chemical Co., Catalog No. 877, or equivalent) in acetate buffer, and dilute to 50 ml. 3. **Borax Buffer**. Dissolve 47.63 g of sodium borate decahydrate ($Na_2B_4O_7•10H_2O$) in warm water. Cool to room temperature. Add 20 ml of 4 N sodium hydroxide (16 g of NaOH dissolved in 100ml water) solution, adjust the pH of the solution to 9.7 with 4 N sodium hydroxide, and dilute to 2000 ml. 4. **p-Nitrophenol Stock Solution.** Dissolve 0.0334 g of p-nitrophenol in water, and dilute to 1000 ml. This solution contains 0.24 µmol/ml. 5. **Standards**: Prepare the following dilutions of p-Nitrophenol Stock Solution with water and read the absorbance at Procedure5 nm

Volume of stock	Volume of DI water	Concentration
100 ml	50 ml	0.16µmol/ml
50 ml	100 ml	0.08 µmol/ml
25 ml	125 ml	0.04 µmol/ml

Created with Print2PDF. To remove this line, buy a license at: http://www.software602.com/

ADVANCED ENZYME TECHNOLOGIES LTD.

A-61/62, MALEGAON MIDC, SINNAR, NASHIK- 422113 (INDIA)

STANDARD ANALYTICAL PROCEDURE

TITLE : α-GALACTOSIDASE METHOD

Sr. No	Test		Method
03	**Preparation Of Enzyme Solutions**	:	Prepare a solution in DI water that contains between 0.001 and 0.003 galactosidase units of activity per ml
04	**Procedure**	:	1. Equilibrate the substrate solution in a water bath at 37° C ± 0.2° C for at least 15 min. 2. Transfer 1.0 ml of each sample to separate test tubes and equilibrate in the 37° C ± 0.2° C water bath. 3. At zero time, add 2.0 ml of substrate solution, mix, and return to the water bath. 4. After exactly 15.0 min, add 5.0 ml of borax buffer to each tube, mix, and remove from the water bath. 5. For sample blanks, transfer in sequence 1.0 ml of each sample to separate test tubes, add 5.0 ml of borax buffer, mix. Add 2.0 ml of substrate solution to each tube, and mix. 6. Measure the absorbance of each standard sample and blank at Procedure5 nm versus that of water. Determine the absorbance of all solutions within 30 min of completing the tests.
05	Calculation	:	Calculate the millimolar extinction of p-nitrophenol standards using the following formula: in which AN is the absorbance of the p-nitrophenol standards at Procedure5 nm and C is the concentration of p-nitrophenol, in µmol/ml The averaged millimolar extinction coefficient should be approximately 18.3. $$\text{Gal U/g} = \frac{(AS - AB) \times 8}{(\in \times 15 \times M)}$$ AS = sample absorbance; AB =blank absorbance; 8 = total volume (ml); 15 = reaction time, in min; M = sample weight, in g/ml; $\in$ = the millimolar extinction coefficient

Created with Print2PDF. To remove this line, buy a license at: http://www.software602.com/