LACTASE (ACID) (β-GALACTOSIDASE) ACTIVITY

(FCC VII)

Application and Principle

This procedure is used to determine lactase activity of enzyme preparations derived from *Aspergillus oryzae var*. The assay is based on a 15-min hydrolysis of an ONPG (o-nitrophenyl-β-D-galactopyranoside) substrate at 37°C and pH 4.5.

Reagents and Solutions

(1.) 2.0 N Acetic Acid: Dilute 57.5 mL of glacial acetic acid to 500 mL with water. Mix well, and store in a refrigerator.

(2.) 4.0 N Sodium Hydroxide: Dissolve 40.0 g of sodium hydroxide in sufficient water to make 250mL.

(3.) Acetate Buffer: Combine 50 mL of 2.0 N Acetic Acid and 11.3 mL of 4.0 N Sodium Hydroxide in a 1000-mL volumetric flask, and dilute to volume with water. Verify that the pH is 4.50±0.05, using a pH meter, and adjust, if necessary, with 2.0 N Acetic Acid or 4.0 N Sodium Hydroxide.

(4.) 2.0 mM o-Nitrophenol Stock: Transfer 139.0 mg of o-nitro-phenol to a 500-mL volumetric flask, dissolve in 10 mL of USP alcohol (95% ethanol) by swirling, and dilute to volume with 1% sodium carbonate.

(5.) 1% sodium carbonate solution: weigh 1g sodium carbonate anhydrous, dissolve to water and dilute to 100ml.

(6.) 10% sodium carbonate solution: weigh 10g sodium carbonate anhydrous, dissolve to water and dilute to 100ml.

o-Nitrophenol Standards

(1.) 0.10 mM Standard Solution: Pipet 5.0 mL of the 2.0 mM o-Nitrophenol Stock solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

(2.) 0.14 mM Standard Solution: Pipet 7.0 mL of the 2.0 mM o-Nitrophenol Stock solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

(3.) 0.18 mM Standard Solution: Pipet 9.0 mL of the 2.0 mM o-Nitrophenol Stock solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

Substrate

Transfer 370.0 mg of o-nitrophenyl-β-D-galactopyranoside to a 100-mL volumetric flask, and add 50 mL of Acetate Buffer. Swirl to dissolve, and dilute to volume with Acetate Buffer.

[**NOTE**—Perform the assay procedure within 2 h of Substrate preparation.]

Test Preparation

Prepare a solution from the test sample preparation such that 1 mL of the final dilution will contain between 0.15 and 0.65 lactase unit. Weigh, and quantitatively transfer the enzyme to a volumetric flask of appropriate size. Dissolve the enzyme in water, swirling gently, and dilute with water if

necessary.
[**NOTE**—Perform the assay procedure within 2 h of dissolution of the Test Preparation.]

System Suitability
Determine the absorbance of the three o-Nitrophenol Standards at 420 nm in a 1-cm cell, using a suitable spectrophotometer. Use water to zero the instrument. Calculate the millimolar extinction, M, for each of the o-Nitrophenol Standards (0.10, 0.14, and 0.18 mM) by the equation:

$$\varepsilon = A_n/C$$

in which A_n is the absorbance of each o -Nitrophenol Standard at 420 nm and C is the corresponding concentration of o-nitrophenol in the standard. M for each standard should be approximately 4.60/mM. Perform a linear regression analysis of the absorbance readings of the three o-Ni-trophenol Standards versus the o-nitrophenol concentration.

in each (0.10, 0.14, and 0.18 mM). There should not be less than 0.99. Determine the mean M of the three o-Nitro-phenol Standards for use in the calculations below.

Procedure:
For each sample or blank, pipet 2.0 mL of Substrate solution into a 25×150-mm test tube, and equilibrate in a water bath maintained at 37.0 °± 0.1° for approximately 10 min. At zero time, rapidly pipet 0.5 mL of the Test Preparation (or 0.5 mL of water as a blank) into the equilibrated substrate, mix by brief (1s) vortex, and immediately return the tubes to the water bath. After exactly 15min of incubation, rapidly add 2.5 mL of 10% sodium carbonate solution, and vortex the tube to stop the enzyme reaction. Dilute the samples and blanks to 25.0 mL by adding 20.0 mL of water, and thoroughly mix. Determine the absorbance of the diluted samples and blanks at 420 nm in a 1-cm cell, using a suitable spectrophotometer. Use water to zero the instrument.

Calculation
One lactase unit (ALU) is defined as that quantity of enzyme that will liberate o-nitrophenol at a rate of 1μmol/min under the conditions of the assay.

Calculate the activity (lactase activity per gram) of the enzyme preparation taken for analysis as follows:

$$ALU/g=[(As-B)(25)]/[(\varepsilon)(15)(W)]$$

in which A_S is the average of absorbance readings for the Test Preparation;
B is the average of absorbance readings for the blank;
25 is the final volume, in milliliters, of the diluted incubation mixture;
E is the mean absorptivity of the o-Ni-trophenol Standards per micromole,
15 is the incubation time, in minutes,
W is the weight, in grams, of original enzyme preparation contained in the 0.5-mL aliquot of Test Preparation used in the incubation.