Alpha Galactosidase (α-Galactosidase) ACTIVITY

(FCC VII)

Application and Principle

Use this procedure to deter-mine 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.

Reagents and Solutions

(1.) Acetate Buffer: dissolve 11.55 mL of glacial acetic acid in water, and dilute to 1 L (Solution A). Dissolve 16.4 g of sodium acetate in water, and dilute to 1 L (Solution B). Mix 7.5 mL of Solution A and 42.5 mL of Solution B, and dilute to 200 mL with water. Adjust the pH of this solution to 5.5 with either Solution A or Solution B as necessary.

(2.) Substrate Solution: dissolve 0.210 g of p-nitrophenyl-α-D-galactopyranoside (Sigma Chemical Co., Catalog No. 877, or equivalent) in and dilute to 100 mL with Acetate Buffer.

(3.) Borax Buffer: dissolve 47.63 g of sodium borate decahydrate in warm water. Cool to room temperature. Add 20mL of 4 N sodium hydroxide solution, adjust the pH of the solution to 9.7 with 4 N sodium hydroxide, and dilute to 2L with water.

(4.) p-Nitrophenol Stock Solution: dissolve 0.0334 g of p-nitro-phenol (Aldrich Chemical Co., Catalog No. 24,132-6, or equivalent) in and dilute to 1 L with water. This solution contains 0.24μmol of p-nitrophenol per milliliter of water.

Preparation of Standards and Samples

Standards:

Prepare the following dilutions of p-Nitrophe-nol Stock Solution with water: 100:50 (v/v) (0.16 μmol/mL); 50:100 (v/v) (0.08 μmol/mL); and 25:125 (v/v) (0.04μmol/mL). Transfer 2.0 mL of the Substrate Solution to each of five separate test tubes. Add 1 mL of the p-Nitrophenol Stock Solution to the first tube, 1.0 mL of each dilution to the next three tubes, and 1.0 mL of water to the fifth tube. Add 5.0 mL of Borax Buffer to each tube, and mix.

Samples:

Prepare a solution of α-galactosidase sample in Acetate Buffer that contains between 0.008 and 0.024 galactosidase units of activity per milliliter.

Procedure:

Equilibrate the Substrate Solution in a water bath at 37°C±0.2°C for at least 15min. For active samples, transfer 1.0 mL of each sample to separate test tubes and equilibrate in the 37°C±0.2°C water bath. At zero time, add 2.0 mL of Substrate Solution, mix, and return to the water bath. After exactly 15.0 min, add 5.0 mL of Borax Buffer to each tube, mix, and remove from the water bath.

For sample blanks, transfer, in sequence, 1.0 mL of each sample to separate test tubes, add 5.0 mL of

Borax Buffer, and mix. Add 2.0 mL of Substrate Solution to each tube, and mix.
Measure the absorbance of each standard sample and blank at 405nm versus that of water. Determine the absorbances of all solutions within 30 min of completing the tests.

Calculation

One galactosidase activity unit (GalU) 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.

Calculate the factor ε for the p-nitrophenol standards using the following equation:

$$\text{Result} = \varepsilon = A_N/C$$

in which A_N is is the absorbance of the p-nitrophenol standards at 405 nm, and C is the concentration, in millimoles per milliliter of p-nitrophenol. Because the averaged millimolar extinction coefficient of p-nitrophenol at 405nm is 18.3, ε should be approximately 2.29 [or (18.3)/8].

$$\text{GalU/g} = [(A_S - A_B) \times F]/(\varepsilon \times T \times M)$$

in which A_S is the sample absorbance; A_B is the blank absorbance; F is the appropriate dilution factor; T is the reaction time, in minutes; M is the weight, in grams, of the sample; and ε is a factor calculated above for the p-nitro-phenol standards (proportional to the millimolar extinction coefficient for p-nitrophenol).

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