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TECHNICAL INFORMATION

Catalog Number: 100780
Lactase

Description: Lactase is an enzyme preparation produced by *Aspergillus oryzae* fermentation. Lactase hydrolyzes Lactose (4-o-b-D-Galactopyranosyl-D-glucose) and produces b-D-Galactose and a-D-Glucose.
Solubility: Soluble in water; Insoluble in ethanol, acetone and isopropyl alcohol.
Stability: Stable in pH range of 4.0 - 8.0. Stable to heat.
Optimum pH Range: Optimum pH's are 4.5 and 4.8 when O-Nitrophenyl-beta-D- galactopyranoside and Lactose are used as substrate, respectively.
Assay Method: A chromogenic substrate ONPG (*o*-Nitrophenyl-b-D-galactopyranoside) is used for the assay.
3.0ml of the substrate solution which contains 200 mg of ONPG per 100 ml of 0.1 M McIlvaine buffer, pH 4.5, is added to 1.0 ml of diluted enzyme solution. After 10 min. incubation of 30°C, reaction is stopped by adding 1 ml of 1M Na_2CO_3 solution. Then the absorbance at 420nm is measured with a spectrophotometer.
Unit Definition: One unit of the lactase activity is defined as the amount of enzyme which produces 1 micromole of o -Nitrophenol per minute under the condition described above.
The lactase activity is also determined from the amounts of liberated glucose. The amounts of glucose are determined by a glucostat method (Glucose oxidase-Peroxidase-Chromogen procedure).
3.5 ml of the substrate solution which contains 172 mg or ONPG per 100 ml of 0.1 M McIlvain buffer, pH4.5, is added to 0.5 ml of diluted enzyme solution. After 10 minutes incubation at 30ºC, the reaction is stopped by adding 1ml of 1 M Na_2CO_3 solution. Then the absorbance at 420 nm is measured with a spectrophotometer.

Notes:

– 0.1 M Na_2HPO_4 is added into 0.1 M citric acid to make pH 4.5.
– Standard curve is made using O-Nitrophenol (yellow color).

Equipment Needed:

– Constant temperature water batch at 37°C ± 0.2°C.
– Cool wate bath (10-15°C).
– Spectrophotometer adjusted to a wave length of 420 nm.
– Stopwatch or timer
– pH meter
– Automatic pipet: 1 ml
– Dispenser: 8 ml
– Dilutor 0.5 ml/10 ml

Reagents:
All of the reagents used must be "analytical grade". Water must be distilled water or equivalent quality.
A. Stock buffer solution pH 4.4
Dissolve 21 g of citric acid monohydrate and 13.6 g of potassium dihydrogenophosphate in 700 ml of water and adjust pH to 4.4 with a concentrated sodium hydroxide solution. Pour into a one liter flask and adjust to mark with water. Store in an amber bottle with a drop or two of toluene as a preservative. Make fresh monthly.
B. Diluted working buffer solution
Dilute 10 ml of the stock solution to 100 ml with water. Check the pH. It should be 4.4. Discard after 2 days.
C. Sodium Carbonate Solution: 10%
Dissolve 50 g of anhydrous sodium carbonate in water and dilute to 500 ml with water. Store in an amber bottle. Make fresh monthly.
D. ONPG Substrate
Dissolve 300 mg of ONPG in 150 ml of water with vigorous agitation. Adjust to 200 ml with water. Unfrozen substrate may be used for 2 days if stored at 4°C. Frozen substrate may be used for several months. Freeze 25 ml aliquotes in polyethylene bottles.
E. O-Nitrophenol Solution (ONP)

1. 0.001 M ONP Solution

Dissolve 139.1 mg of ONP in 50 ml of ethanol and complete to 1000 ml with water.
2. Diluted working solution
Dilute 20.0 ml of the 0.001 M ONP solution to 200.0 ml with water; 1 ml of this solution contains 0.1 micromole of ONP.

Procedure:
A. O-Nitrophenol Standard Curve

Option #1: Into a series of 18 x 180 test tubes pipet respectively 0, 2, 4, 6, 8, 10 ml of the diluted working solution of ONP and complete to 10 ml with water.
Option #2: If a dilutor is used, pipet respectively 0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of the 0.001 M stock solution of ONP and dilute with respectively 10, 9.8, 9.6, 9.4, 9.2, 9.0 ml of water.

Add to each tube 1 ml of the carbonate solution and mix. Each tube contains respectively 0, 0.2, 0.4, 0.6, 0.8, 1.0 micromole of ONP.
Read absorbances at 420 nm with a spectrophotometer, using as a reference the tube without ONP.
On graph paper plot the quantities of ONP per tube on abscissa and the corresponding absorbances on ordinate.
Draw a line through the best average of the plotted points. A straight line should result.

B. Enzymatic Solutions
Prepare by diluting the enzyme with water so that the solutions contain between 2.0 and 3.0 units/ml.
C. Enzymatic Action
Prepare 3 test tubes (18 x 180) per enzymatic solution to be tested (one blank and two assays). Mark these tubes T, E_1, and E_2.
Measure 8.0 ml of the working buffer into each tube; add 1.0 ml of ONPG substrate and place tubes E_1 and E_2 immediately in the 37°C waterbath. Wait for temperature equilibrium (not more than 10 minutes).
At zero time (using a stopwatch or timer) add 1 ml of the enzymatic solution to the tubes E_1 and E_2. Mix and replace in the 37°C waterbath.
After exactly 20 minutes incubation, add 1 ml of the carbonate solution to E_1 and to E_2. Mix and place these tubes in the cool waterbath. Add 1 ml of the carbonate solution, the 1 ml of the enzymatic solution the tube T placed in the cool waterbath.
Read the absorbances of E_1 and E_2 against the blank T with a spectrophotometer of 420 nm using 1 cm glass cuvet. Read within 30 minutes following the addition of the carbonate.
With each series of determinations of unknown samples, assay a standard lactase of known activity.
Calculation of the Lactase (beta-Galactosidase) Activity
Reading of E_1 and E_2 give respectively absorbances A_1 and A_2.

$$\text{Calculate the mean} \quad A = \frac{A_1 + A_2}{2}$$

A1 and A2 should not differ more than 2.5%. From the ONP standard curve determine the quantity (x), in micromoles, of ONP that corresponds to A.
Calculate the enzymatic activity

$$LA/g = \frac{5X}{C}$$

where: X = micromoles of ONP (from the standard curve).
C = Concentration of enzyme solution (g/ml).