Megazyme

ASSAY OF

I,6-ALPHA-DEXTRAZYME

using

ALPHA-DEXTRAZYME TABLETS

T-DEXT 02/03

SUBSTRATE:

The substrate employed is azurine-crosslinked dextran T-2000 (AZCL-Alpha-Dextran). The substrate is prepared by dyeing and crosslinking dextran T-2000 to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-(1,6)-α-dextranase (dextranase) produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity. The substrate is supplied commercially in a ready-to-use tablet form as **Alpha-Dextrazyme Tablets** (containing AZCL-Dextran).

BUFFER STOCK SOLUTION:

(Sodium Acetate buffer, 1M, pH 4.5)

Glacial acetic acid (60.0 g, 1.05 g/mL) is added to 800 mL of distilled water. This solution is adjusted to pH 4.5 by the addition of 5 M (20 g/100 mL) sodium hydroxide solution. The volume is then adjusted to 1 litre. Store at room temperature

EXTRACTION/DILUTION BUFFER:

(Sodium acetate, 100 mM, pH 4.5) containing sodium azide (0.02%) and BSA (0.5 mg/mL)

Buffer stock solution (100 mL) is added to 850 mL of distilled water. The pH is adjusted to pH 4.5 by dropwise addition of 2 M hydrochloric acid solution. Sodium azide (0.2 g) is added and dissolved, and the volume is adjusted to 1 litre. BSA (0.5 g) is added and dissolved. This buffer is stable for 12 months at 4°C.

NOTES:

1. When preparing the extraction/dilution buffer, do not add the **sodium azide** until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.
2. In the assay format described here, **a single blank** is required for each set of determinations and this is used to zero the spectrophotometer. The absorbance of the reaction solutions are read against this blank.

ENZYME EXTRACTION AND DILUTION:

Liquid enzyme sample (1.0 mL) is added, using a positive displacement dispenser (these solutions can be very viscous), to **extraction/dilution buffer** (49 mL, pH 4.5) and mixed thoroughly. This is termed the **Original Extract**. An aliquot of this solution (1.0 mL) is then diluted 10-fold by addition to 9 mL of **extraction/ dilution buffer**. This process of dilution is repeated until a suitable dilution of the enzyme preparation is achieved. BSA is added to stabilise the *endo*-1,6-α-dextranase on dilution. With powder samples, the preparation (1.0 g) is added to **extraction/dilution buffer** (50 mL, pH 4.5) and the slurry is gently mixed over a period of approximately 15 min or until the sample is completely dispersed or dissolved. This solution (the **Original Extract**) is clarified by centrifugation (1,000 *g*, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. This extract is then diluted further with **extraction/dilution buffer**, as for the liquid enzyme samples.

ASSAY PROCEDURE:

1. Pre-equilibrate aliquots (0.5 mL) of suitably diluted enzyme preparation (in extraction/dilution buffer) at 40°C for 5 min in glass test tubes (16 x 120 mm).
2. Initiate the reaction by the addition of a **1,6-Alpha-Dextrazyme** tablet. The tablet hydrates rapidly. The suspension **should not** be stirred. Incubate the tube at 40°C for 10 min.
3. Terminate the reaction after exactly 10 min at 40°C by adding Trizma Base solution (10.0 mL, 2% w/v) with vigorous stirring on a vortex mixer.
4. Leave the tubes at room temperature for 4-5 min, stir the slurry and then filter through a Whatman No. 1 (9 cm) filter circle.
5. Measure the absorbance of the filtrate at 590 nm against a **substrate/enzyme blank**. The **substrate/enzyme blank** is prepared by adding Trizma Base solution (10.0 mL, 2% w/v) to the enzyme solution before the addition of the Alpha-Dextrazyme tablet. This slurry **must** be left at room temperature. A single blank is required for each set of determinations and this is used to zero the spectrophotometer.

STANDARDISATION:

A Standard curve relating the activity of highly purified *Penicillium lilacinum endo*-1,6-α-dextranase from the commercial enzyme preparation Dextranase 50 L (from Novo Nordisk) on dextran B-512 and 1,6-Alpha-Dextrazyme Tablets (Lot 60901) is shown in Figure 1. Activity on dextran B-512 was determined at a substrate concentration of 10 mg/mL in 100 mM sodium acetate buffer (pH 4.5) at 40°C using the Nelson/Somogyi reducing sugar method.

One **Unit** of activity is defined as the amount of enzyme required to release one micromole of glucose reducing-sugar-equivalents per minute from dextran B-512 (Somogyi reducing sugar method) at pH 4.5 and 40°C.

CALCULATION OF ACTIVITY:

***endo*-1,6-α-Dextranase** activity is determined by reference to the standard curve to convert absorbance to milliUnits of activity per assay on dextran, and then calculated as follows:

Units/mL or gram of Original Preparation:

$$= \text{milliUnits (per assay i.e. per 0.5 mL)} \times 2 \times 50 \times \frac{1}{1000} \times \text{Dilution}$$

where:

2 = conversion from 0.5 mL to 1.0 mL.

50 = the volume of buffer used to extract the original preparation (i.e. 1 g/50 mL or 1.0 mL of enzyme added to 49 mL of buffer).

$\frac{1}{1000}$ = conversion from milliUnits to Units.

Dilution = further dilution of the Original Extract.

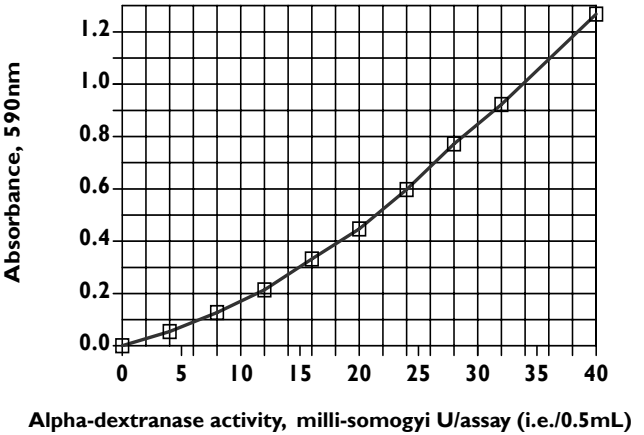

Figure 1. *Penicillium lilacinum endo*-1,6-α-dextranase standard curve on 1,6-Alpha-dextrazyme (Lot 60901).

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