Megazyme

ASSAY OF CHITOSANASE

USING

CHITOZYME TABLETS

CHZ 01/04

SUBSTRATE:

The substrate employed is Azurine-crosslinked chitosan (AZCL-Chitosan). AZCL-Chitosan is a simple and very specific substrate for the assay of **chitosanase** and can be used to measure this activity in the presence of large excesses of other enzymes and in the presence of sugars. This substrate is supplied commercially in a ready-to-use form as Chitozyme tablets.This assay is best described as semi-quantitative and is useful for research purposes,but is not ideal for quantitative assays.

EXTRACTION/DILUTION BUFFER:

[(Sodium Acetate buffer, 200 mM,pH 4.5) containing sodium azide (0.02%)]

Add 12.2 g of glacial acetic acid (1.05 g/ml) to 900 ml of distilled water. Adjust the pH of this solution to pH 4.5 by the addition of 1 M (4 g/100 ml) sodium hydroxide solution. (Approximately 50 ml is required). Add 0.2 g of sodium azide and adjust the volume to 1 litre. Store at 4°C.

ENZYME DILUTION:

Dilute enzyme preparations in 200 mM sodium acetate buffer (pH 4.5).

NOTE: Sodium azide is a toxic chemical and should be treated accordingly.

ASSAY PROCEDURE:

1. Equilibrate 0.5 ml aliquots of suitably diluted enzyme in extraction/ dilution buffer at 40°C for 5 min.

2. Initiate the reaction by the addition of a Chitozyme tablet. The tablet hydrates rapidly. The suspension should not be stirred.

3. Terminate the reaction after exactly 10 min at 40°C by the addition of 10 ml of Trizma Base solution (2 % w/v, pH ~ 8.5) (Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer.

4. After about 5 min standing at room temperature, stir the slurry again and filter through a Whatman No.1 (9 cm) filter circle.

5. Measure the absorbance of the reaction solutions at 590 nm against the reaction blank.

If the absorbance value is greater than 0.6 Units ,dilute the enzyme solution further as required,and repeat the assay.

Prepare a substrate/enzyme (reaction) blank by adding 10 ml of Trizma Base to the enzyme solution (0.5 ml) before the addition of the Chitozyme tablet.

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 purified chitosanase on chitosan and Chitozyme tablets (Lot 30101) is shown in Figure 1. The curve obtained for chitosanase in a crude commercial enzyme preparations was the same. Activity on purified chitosan was determined at a substrate concentration of 5 mg/ml in 200 mM sodium acetate buffer (pH 4.5) at 40°C using the Nelson/Somogyi reducing sugar procedure and a glucosamine standard.

CALCULATION OF ACTIVITY:

1. Chitosanase activity in the sample being assayed is determined by reference to the standard curve to convert absorbance values to milliUnits per assay (i.e. per 0.5 ml).

2. ***Chitosanase* activity per ml or gram of original preparation:**

$$= \text{milliUnits/assay} \times \frac{1}{1000} \times 2 \times \text{Dilution}$$

where:

2 = conversion from milliUnits to units.

$\frac{1}{1000}$ = conversion from 0.5 ml to 1.0 ml.

Dilution = the dilution of the original enzyme preparation

One **Unit of activity** is defined as the amount of enzyme required to release one micromole of glucosamine reducing-sugar equivalents from chitosan (5 mg/ml) per minute under the defined assay conditions (40°C and pH 4.5).

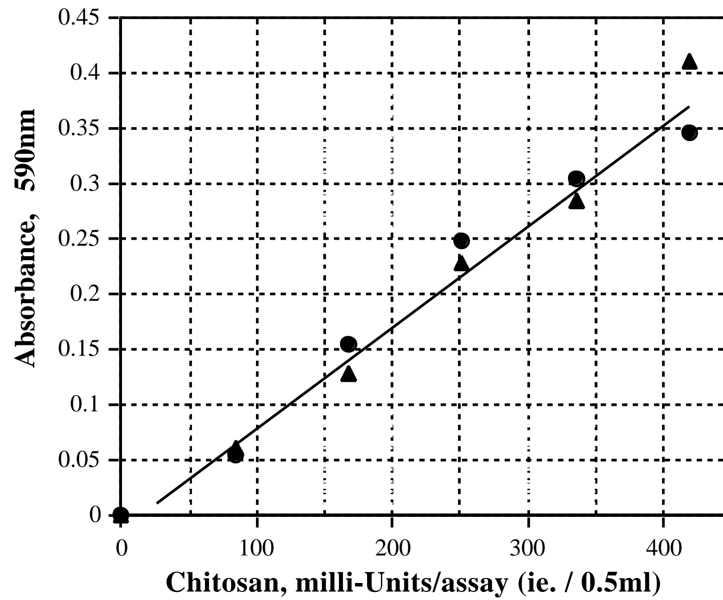

Figure 1. Chitosanase standard curve on Chitozyme tablets (Lot 30101).

Chitosanase (0.5 ml, 0-500 milliUnits) in 200 mM sodium acetate buffer (pH 4.5) in a glass test-tube (16 x 120 mm) was pre-equilibrated at 40°C for 5 min. The reaction was initiated by the addition of a Chitozyme tablet without stirring. Reaction was terminated after 10 min by the addition of 10 ml of Trizma Base (2% w/v) with vigorous stirring. After about 5 min the tubes were stirred again, and the solutions were filtered through Whatman No. 1 (9 cm) filter circles. The absorbance of the filtrates were read against an enzyme/substrate blank solution at 590 nm.

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