

Megazyme

ASSAY OF LIMIT-DEXTRINASE
IN CEREAL FLOURS

THE LIMIT-DEXTRIZYME METHOD

LDZ 7/98



SUBSTRATE:

The substrate is Azurine-crosslinked-pullulan, which is hydrolysed by limit-dextrinase and pullulanase, but is resistant to attack by other commonly occurring amylolytic enzymes such as α -amylase (cereal and bacterial), β -amylase and amyloglucosidase. The substrate is slowly hydrolysed (and to a limited extent) by *A. niger* α -amylase. This enzyme can act at the α -1,4-maltotetraosyl structural units in pullulan. The substrate is prepared by dyeing and crosslinking pullulan polysaccharide to produce a material which hydrates in water, but is water insoluble. Hydrolysis by limit-dextrinase (pullulanase) 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 Limit-Dextrizyme.

BUFFERS:

1. Buffer A: Dilution Buffer

[Sodium maleate (100 mM, pH 5.5) plus sodium azide (0.02%).]

Dissolve maleic acid (5.8 g, Sigma Chemical Co., cat. no. M-0375) in 400 mL of distilled water and adjust the pH to 5.5 with sodium hydroxide solution (2 M) (requires about 35 mL). Add sodium azide (0.1 g) and readjust the pH to 5.5. Adjust the volume to 500 mL. Store at room temperature.

NOTE: Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas. Sodium azide can be deleted from the buffer if desired, but then the buffer should be stored at 4°C between use.

2. Buffer B: Extraction/Activation Buffer

[Sodium maleate (100 mM, pH 5.5) plus sodium azide (0.02%)] plus 25 mM dithiothreitol (for extraction and activation of malt limit-dextrinase).]

Immediately before use, add dithiothreitol (0.1g) to 25 mL of Buffer A.

NOTE: Dithiothreitol is required for complete extraction and activation of malt limit-dextrinase. Buffer B should be prepared immediately before use.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (16 x 120 mm, ;17 mL capacity, round bottomed).
2. Micro-pipettors, 0.5 mL (e.g. Gilson Pipetman[®]) (for enzyme preparation).
3. Adjustable-volume dispenser (for Trizma base solution).
4. Bench centrifuge (required speed 3,000 rpm), or Whatman GF/A glass fibre filter paper.
5. Analytical and top-pan balances.
6. Spectrophotometer set at 590 nm.
7. Vortex mixer (e.g. Thermolyne Maxi Mix II).
8. Thermostatted water bath set at 40.0°C (e.g. Julabo PC).
9. Stop clock.
10. Whatman No. 1 (9 cm) filter circles and filter funnels.

CONTROLS AND PRECAUTIONS:

1. The time of incubation of the enzyme extract with the Limit-Dextrizyme must be carefully controlled (i.e. 10.0 min).
2. Incubation temperatures must be accurately controlled (i.e. 40.0°C).
3. After addition of Trizma base to the reaction tube, the tubes must be stirred vigorously to ensure thorough mixing.
4. On addition of the Trizma base solution to the reaction tubes, the tubes must be stored at room temperature. Under these conditions, the substrate is stable for several hours. Storage of the substrate suspension under alkaline conditions at elevated temperatures will lead to slow hydrolysis and release of dye molecules, resulting in elevated blank and/or reaction values.
5. With each set of analyses a single reaction blank should be run. This is performed by adding a Limit-Dextrizyme tablet to the appropriate buffer and proceeding as for the enzyme assays.
6. Test tablets should be stored dry in well sealed containers at room temperature.
7. The Limit-Dextrizyme assay is affected by the concentration of buffer salts. The optimal buffer salt concentration is 100 mM.

ENZYME EXTRACTION FROM CEREAL FLOURS:

Grain or malt samples are milled to pass a 0.5 mm mesh.

Flour samples (0.25 g) are suspended in Buffer B (4.0 mL) and allowed to extract at 40°C over a period of 5-6 hours. Over this time period, the tubes are sealed and the slurries are stirred on a test-tube stirrer 4-5 times and then centrifuged at 1,000 *g* (approx. 3,000 rpm) for 10 min.

Aliquots (0.5 mL) for assay can be taken directly from the centrifuge tubes, or alternatively, the supernatants can be poured off, stored, and assayed for activity within the next 2-3 hours.

ASSAY PROCEDURE:

1. An aliquot (0.5 mL) of flour extract or enzyme solution is pre-equilibrated at 40°C for 5 min in a glass test-tube (16 x 120mm).
2. Reaction is initiated by the addition of a Limit-Dextrizyme tablet. The tablet rapidly hydrates and disintegrates within 20 seconds. The suspension should not be stirred.
3. After exactly 10 min, the reaction is terminated by the addition of Trizma Base solution (5 mL, 1% w/v, Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer. The tubes are left at room temperature.
4. After about 5 min the slurry is stirred again and then filtered through a Whatman No. 1 (9 cm) filter circle.
5. The absorbance at 590 nm of the filtrate is measured against the reaction blank.

A substrate/enzyme blank is prepared by adding Trizma Base to the enzyme solution before the addition of the Limit-Dextrizyme tablet.

NOTES:

1. The Limit-Dextrizyme tablets now supplied by Megazyme are 60% of the weight of tablets previously supplied. Consequently reagent volumes have been adjusted.
2. A single blank is required for each set of determinations. The absorbance of this blank and of the reaction solutions are measured at 590 nm against distilled water. The blank absorbance value is subtracted from the reaction values before reference to the standard curve.
3. After addition of Trizma Base, tubes should not be stored at temperatures above room temperature, and the slurry should be filtered within 30 min. The dye-carbohydrate covalent linkage is slowly cleaved under alkaline conditions at elevated temperatures.

STANDARDISATION:

A standard curve relating the activity of purified malt flour limit-dextrinase on pullulan and Limit-Dextrizyme (Lot 40801) is shown in Fig. 1. The curve will vary slightly with different lots of tablets. Please ensure that the Limit-Dextrizyme lot numbers on the Standard Curves and the Tablet Pack are the same. The curve obtained for rice limit-dextrinase is identical to that obtained with the malt flour enzyme. Activity on pullulan was determined at a substrate concentration of 5 mg/mL in 100 mM maleate buffer (pH 5.5) at 40°C using the Nelson/ Somogyi reducing sugar procedure.

One Unit of activity is defined as the amount of enzyme required to release one micromole of glucose reducing-sugar equivalents per minute from pullulan under the defined assay conditions.

The standard curve relating limit-dextrinase activity to absorbance (590 nm) is not linear.

CALCULATION OF ACTIVITY:

Malt Limit-Dextrinase activity is determined by reference to the standard curve to convert absorbance to milliUnits per assay, and then calculated as follows:

Units/kg Malt

$$= \text{milliUnits per assay (i.e. per 0.5 mL)} \times \frac{1}{1000} \times 32,000$$

where:

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

$$32,000 = \text{conversion from activity/0.5 mL of extract to that in 1 kg of malt. Flour is extracted with 16 ml of buffer per gram of flour, and the assay is performed on 0.5 ml.}$$

REFERENCE:

McCleary, B.V. (1992). "Measurement of the content of limit-dextrinase in cereal flours". *Carbohydrate Research*, **227**, 257-268.

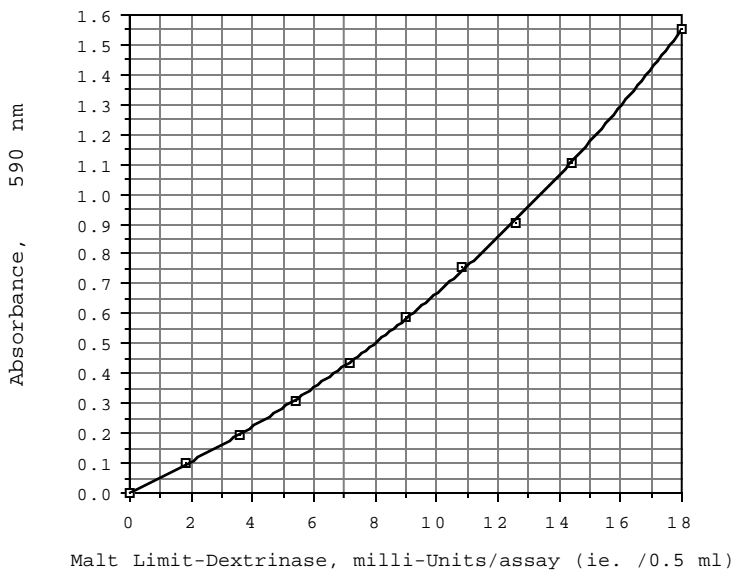


Figure 1. Malt limit-dextrinase standard curve on Limit-Dextrizyme (Lot 40801).



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