Megazyme

ASSAY OF

***endo*-1,3-Beta-Glucanase**

using

1,3-BETA-GLUCAZYME HS TABLETS

T-CUR200 03/12

SUBSTRATE:

The substrate employed is Azurine-crosslinked curdlan (AZCL-Curdlan). The substrate is prepared by dyeing and crosslinking highly purified curdlan to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-1,3-β-glucanase 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 1,3-Beta-Glucazyme HS Tablets.

STOCK BUFFER A:
(Sodium acetate buffer, 2M, pH 4.5)

Add 114 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH to 4.5 by the addition of 4 M (16 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 litre.

EXTRACTION/DILUTION BUFFER A:
[Sodium acetate, 100 mM, pH 4.5, plus BSA (0.5 mg/mL) and sodium azide (0.02%)]

Add 50 mL of stock buffer solution to 850 mL of distilled water and adjust the pH to 4.5. Add 0.2 g of sodium azide and 0.5 g of bovine serum albumin (BSA; Sigma A2153-100G) and dissolve. Adjust the volume to 1 litre. Store at 4°C.

NOTE: Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.

STOCK BUFFER B:
(Sodium acetate buffer, 2M, pH 5.0)

Add 114 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH 5.0 by the addition of 4 M (16 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 litre.

EXTRACTION/DILUTION BUFFER B:
[Sodium acetate, 100 mM, pH 5.0, plus BSA (0.5 mg/mL) and sodium azide (0.02%)]

Add 50 mL of stock buffer solution to 850 mL of distilled water and adjust the pH to 5.0. Add 0.2 g of sodium azide and 0.5 g of bovine serum albumin (BSA; Sigma A2153-100G) and dissolve. Adjust the volume to 1 litre. Store at 4°C.

EXTRACTION/DILUTION BUFFER C: [Sodium phosphate, 100 mM, pH 6.5, plus BSA (0.5 mg/mL) and sodium azide (0.02%)]

Add 17.8 g of di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) to 900 mL of distilled water and dissolve by stirring. Adjust the pH to 6.5 with 1 M HCl. Add 0.2 g of sodium azide and 0.5 g of bovine serum albumin (BSA; Sigma A2153-100G) and dissolve. Adjust the volume to 1 litre with distilled water. Store at 4°C.

ENZYME EXTRACTION AND DILUTION:

A. Cereal Flours and Malt Extracts:

Add 0.50 g of milled malt (milled to pass a 0.5 mm screen) to a glass centrifuge tube (16 x 120 mm). Add 5.0 mL of Extraction/Dilution Buffer B (pH 5.0) and stir the contents thoroughly on a vortex mixer.

Allow the enzyme to extract over 15 min at room temperature (at less than 30°C), with occasional mixing. Centrifuge the tube and contents at 1,000 *g* for 10 min, or filter the slurry through glass fibre filter paper (e.g. Whatman GF/C).

B. Microbial Enzyme Preparations:

Liquid preparations:

Using a positive displacement dispenser, add 1.0 mL of liquid enzyme preparation to a 100 mL volumetric flask and dilute to volume with Extraction/Dilution buffer A, B or C (depending on the pH optima of the particular enzyme) and mix thoroughly. Further dilute this solution by adding 1.0 mL to 9.0 mL of the appropriate Extraction/Dilution buffer with thorough mixing. Dilute further with appropriate Extraction/Dilution buffer as required for assay.

Powder preparations:

Add 1.0 g of powder sample to 90 mL of 100 mM Extraction/Dilution buffer A, B or C and mix until either completely dissolved or dispersed, and adjust to 100 mL. Clarify an aliquot of this solution by centrifugation (1,000 g, 10 min) or filtration through a Whatman No.1 filter circle. Dilute further with appropriate Extraction/Dilution buffer, as required for assay.

ASSAY PROCEDURE:

A. *endo*-1,3-β-Glucanase in Cereal Flours and Malt Extracts:

1. Pre-equilibrate an aliquot (0.5 mL) of suitably diluted and buffered enzyme preparation in a 16 x 120 mm glass test tube at 30°C for 5 min.

2. Add a 1,3-Beta-Glucazyme HS tablet to the tube without stirring. The tablet hydrates rapidly. Incubate at 30°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of Trizma Base solution (2% w/v, pH ~ 8.5) with vigorous stirring on a vortex mixer.
4. Leave the tubes at room temperature for about 5 min, and then stir them again.
5. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
6. Measure the absorbance of the filtrate at 590 nm against a substrate blank.

If the absorbance is above 2.0, dilute an aliquot of the enzyme extract with an equal volume of extraction/dilution buffer and repeat the assay.

Prepare the **substrate blank** by adding a 1,3-Beta-Glucazyme HS tablet to 0.5 mL of extraction buffer. Incubate at 30°C for 10 min and add 10.0 mL of Trizma Base (2 % w/v). Stir the tube contents and after 5 min, filter through a Whatman No. 1 (9 cm) filter paper.

NOTE: A single blank is required for each set of determinations and this is used to zero the spectrophotometer.

B. *endo*-1,3-β-Glucanase in Microbial Preparations:

1. Pre-equilibrate an aliquot (0.5 mL) of suitably diluted and buffered enzyme preparation in a 16 x 120 mm glass test tube at 40°C for 5 min.
2. Add a 1,3-Beta-Glucazyme HS tablet to the tube without stirring. The tablet hydrates rapidly. Incubate at 40°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of Trizma Base solution (2% w/v, pH ~ 8.5) with vigorous stirring on a vortex mixer.
4. Proceed with the assay as for *endo*-1,3-β-glucanase from cereal flours and malt extracts from Step 4.

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

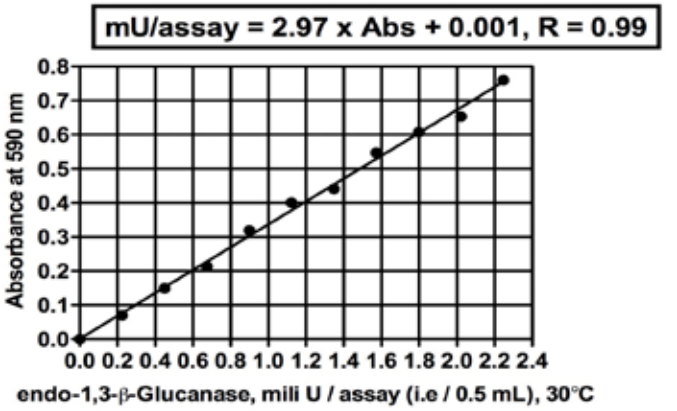

Figure 1. Standard curve for the action of *endo*-1,3-β-glucanase from barley (E-LAMHV; cloned barley gene for *endo*-1,3-β-glucanase) on 1,3-Beta-Glucazyme HS Tablets (Lot 110802). The *endo*-1,3-β-glucanase was standardised on CM-Curdlan (DS = 0.3; Lot 120201a). Both assays performed in Extraction / Dilution buffer B (pH 5.0) at 30°C.

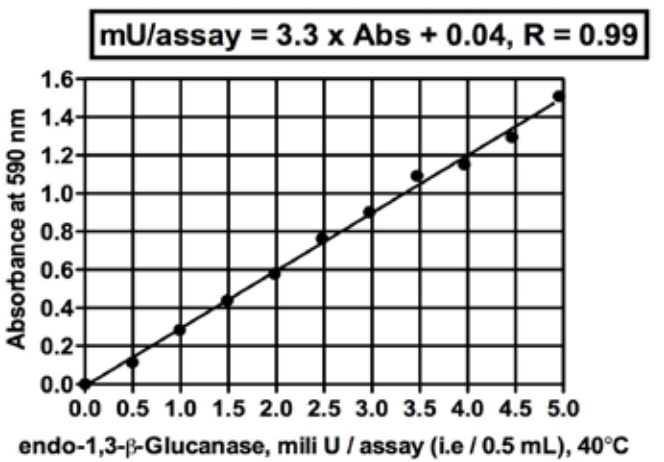

Figure 2. Standard curve for the action of *endo*-1,3-β-glucanase from barley (E-LAMHV; cloned barley gene for *endo*-1,3-β-glucanase) on 1,3-Beta-Glucazyme HS Tablets (Lot 110802). The *endo*-1,3-β-glucanase was standardised on CM-Curdlan (DS = 0.3; Lot 120201a). Both assays performed in Extraction / Dilution buffer B (pH 5.0) at 40°C.

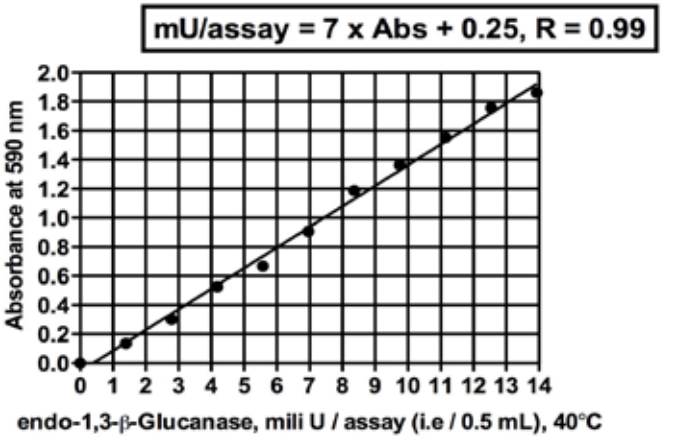

Figure 3. Standard curve for the action of *endo*-1,3(4)-β-glucanase from *Clostridium thermocellum* (E-LICACT) on 1,3-Beta-Glucazyme HS Tablets (Lot 110802). The *endo*-1,3-β-glucanase was standardised on CM-Curdlan (DS = 0.3; Lot 120201a). Both assays performed in Extraction / Dilution buffer C (pH 6.5) at 40°C.

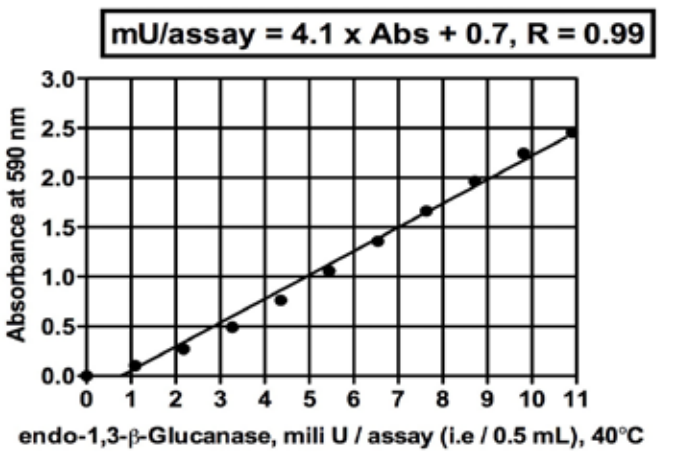

Figure 4. Standard curve for the action of *endo*-1,3-β-glucanase from *Trichoderma* sp. (E-LAMSE) on 1,3-Beta-Glucazyme HS Tablets (Lot 110802). The *endo*-1,3-β-glucanase was standardised on CM-Curdlan (DS = 0.3; Lot 120201a). Both assays performed in Extraction / Dilution buffer A (pH 4.5) at 40°C.

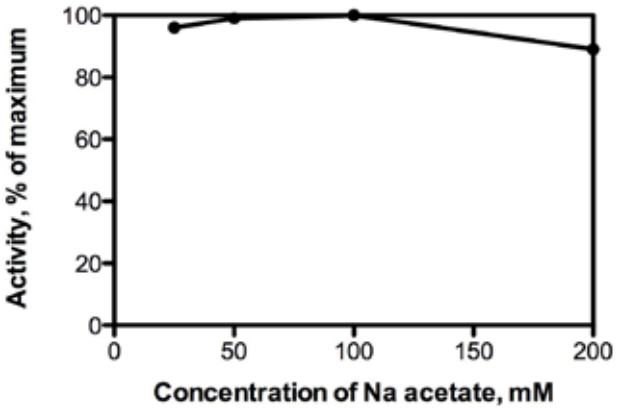

Figure 5. Effect of buffer salt concentration on action of *endo*-1,3-β-glucanase (E-LAMSE) on 1,3-Beta-Glucazyme HS tablets (Lot 110802) at 40°C.

Table 1. Relative rate ***Figure 5.*** Effect of buffer salt concentration on action of *endo*-of action of *endo*-1,3-β-glucanases on laminarin (Sigma Cat. No. L9634-5G; Lot 108K3791), CM-Curdlan (Megazyme Lot 120201a) and CM-Pachyman (Megazyme Lot 100401).

Substrate	Enzyme		
	E-LAMSE	E-LAMHV	E-LICACT
CM- Curdlan (DS - 0.3)	100	100	100
CM –Pachyman (DS -0.2)	81	98	26
Laminarin	47	346	599

In Figures 1-4, the action of *endo*-1,3-β-glucanase was standardised using CM-Curdlan (DS = 0.3; Lot 120201a). From Table 1, it is evident that the enzymes vary significantly in their ability to hydrolyse various 1,3-β-glucans. This difference is due mainly to the effect of substitution by carboxymethyl groups on the susceptibility of the 1,3-β-glucan to hydrolysis by the enzyme. If activity on a substrate other than CM-Curdlan (DS = 0.3) is required, then the relative activities detailed in Table 1 can be used to calculate this.

Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com