endo-beta-Mannanase Microplate Assay Kit User Manual

Catalog # ASK1189

Detection and Quantification of endo-beta-Mannanase Activity in Tissue extracts, Cell lysate, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

endo-beta-Mannanase (EC 3.2.1.78), also known as Mannan endo-1,4-beta-mannosidase, is an enzyme with systematic name 4-beta-D-mannan mannanohydrolase. This enzyme catalyses the following chemical reaction: Hydrolysis of (1->4)-beta-D-mannosidic linkages in mannans, galactomannans and glucomannans. This cleavage occurs at random internal sites within the chain. endo-beta-Mannanase Microplate Assay Kit is a sensitive assay for determining endo-beta-Mannanase activity in various samples. endo-beta-Mannanase hydrolyzes the mannan to generate mannose. Mannose react with 3,5-dinitrosalicylic acid to generate red-brown substance. The color intensity, measured at 540 nm, is proportionate to the enzyme activity in the sample.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml Assay Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, then add 100 µl into 900 µl distilled water; the concentration will be 3 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven

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IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	--	--	--
Assay Buffer	--	20 µl	--	--
Substrate	80 µl	80 µl	--	--
Mix, put it into the oven, 37 °C for 10 minutes.				
Supernatant	100 µl	100 µl	--	--
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put the microplate into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.				

Note: if the sample's activity is higher, please reduce reaction time in the first incubate step.

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VI. CALCULATION

Unit Definition: One unit of endo-beta-Mannanase activity is the enzyme generates 1 μmol of reducing sugar per minute.

1. According to the protein concentration of sample

$$\text{endo-beta-Mannanase (U/mg)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{endo-beta-Mannanase (U/g)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{endo-beta-Mannanase (U/10}^4\text{)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of Standard, 3 mmol/L = 3 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.1 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 10 minutes.

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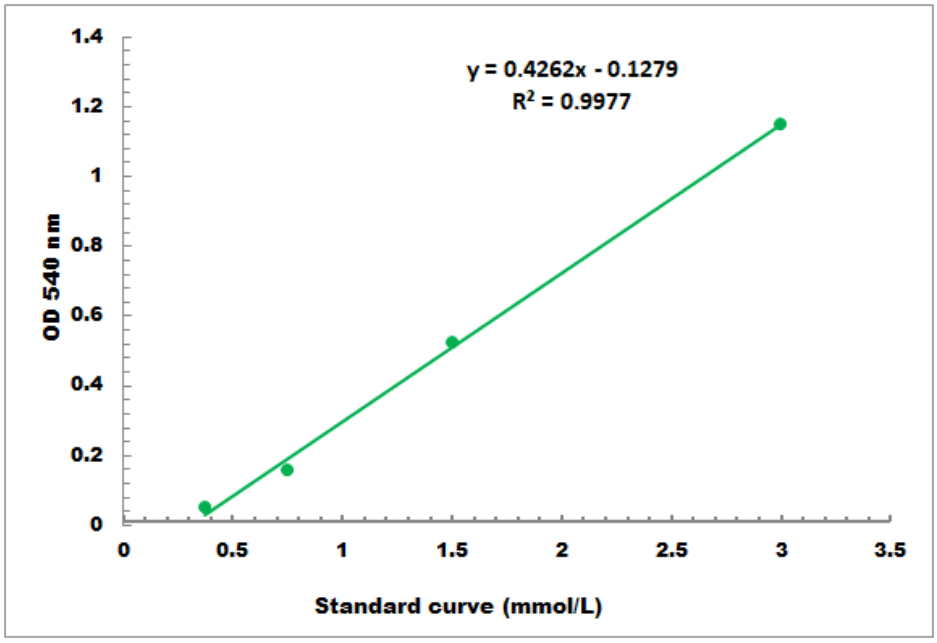
VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.3 mmol/L - 3 mmol/L

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