endo-beta-Mannanase

Microplate Assay Kit

User Manual

Catalog # CAK1189

(Version 1.2C)

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

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

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-Mannanaseactivity in various samples.endo-beta-Mannanase hydrolyzes the mannan to generate mannose. Mannosereact 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.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent	10 mlx 1	4 °C
Standard	Powderx 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

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

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 10mmol/L.

Positive Control: add 0.1 ml assay bufferto dissolve before use, mix.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

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

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of standard, 10mmol/L = 10μmol/ml;

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

W: the weight of sample, g;

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

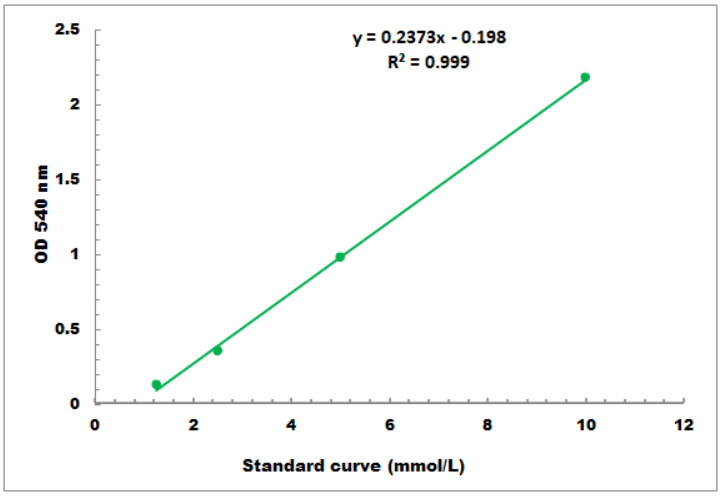
V_{Sample}: the volume of sample, 0.02 ml;

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

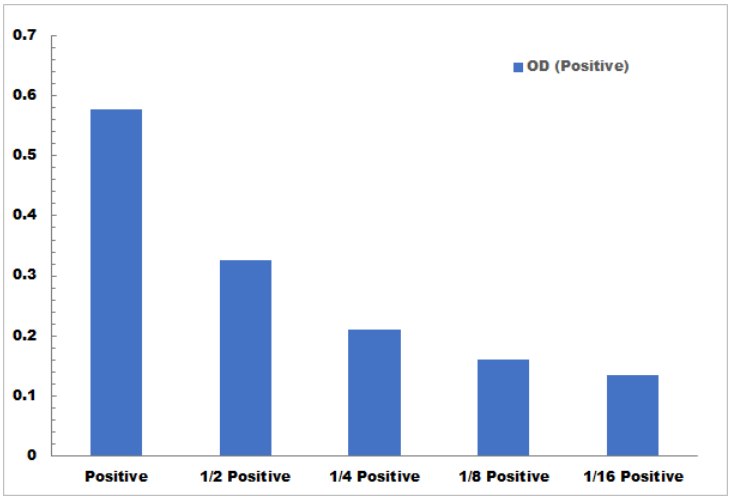
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 1mmol/L -10mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES