endo-beta-Mannanase Activity ColorimetricMicroplate Assay Kit User Manual

Catalog # CAK1189

(Version 1.3C)

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 Activity Colorimetric 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	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 10 mmol/L of standardtop solution, store at 4 °C for 3 monthsafter reconstitution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Substrate: Add 8 ml Assay Buffer to dissolve before use.Store at 4 °C for 1-2 weeks or -20°C for 5-6months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 ml Assay Bufferto dissolve before use, mix.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

$V_{Standard}$: the volume of standard in assay procedure, mL

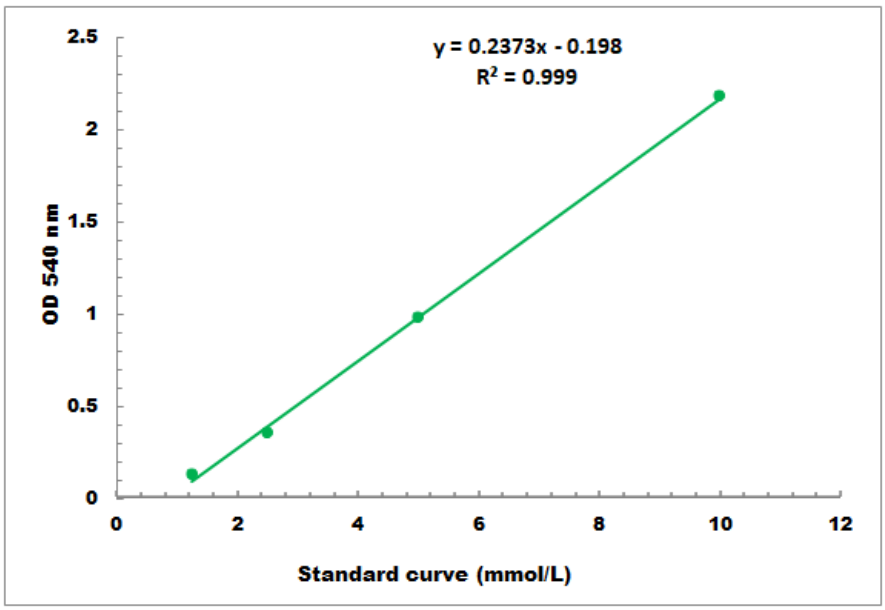
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

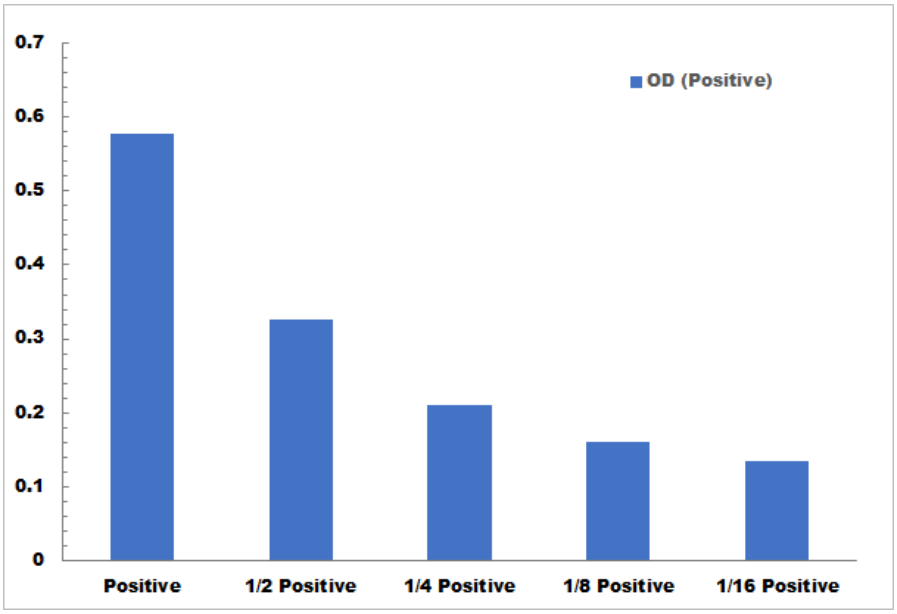
T: the reaction time, minute

VIII. 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