

endo-beta-Mannanase

Microplate Assay Kit

Cat #: orb545639 (manual)

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.

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 catalyzes 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 reacts 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.

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
Positive Control	Powder x 1	-20 °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, the concentration will be 10 mmol/L.

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

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

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%, sonicate 3s, interval 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.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	20 μ l	--	--	--	--
Assay Buffer	--	20 μ l	--	--	--
Positive Control	--	--	--	--	20 μ l
Substrate	80 μ l	80 μ l	--	--	80 μ l
Mix, put it into the oven, 37 °C for 10 minutes.					
Standard	--	--	100 μ l	--	--
Distilled water	--	--	--	100 μ l	--
Dye Reagent	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l
Mix, put the microplate into the convection oven, 90 °C for 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.

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

$$\begin{aligned}\text{endo-beta-Mannanase (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times C_{\text{Protein}}) / T \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{endo-beta-Mannanase (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cell or bacteria

$$\begin{aligned}\text{endo-beta-Mannanase (U/10}^4\text{)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

C_{Standard} : the concentration of standard, 10 mmol/L = 10 $\mu\text{mol/ml}$;

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

W: the weight of sample, g;

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

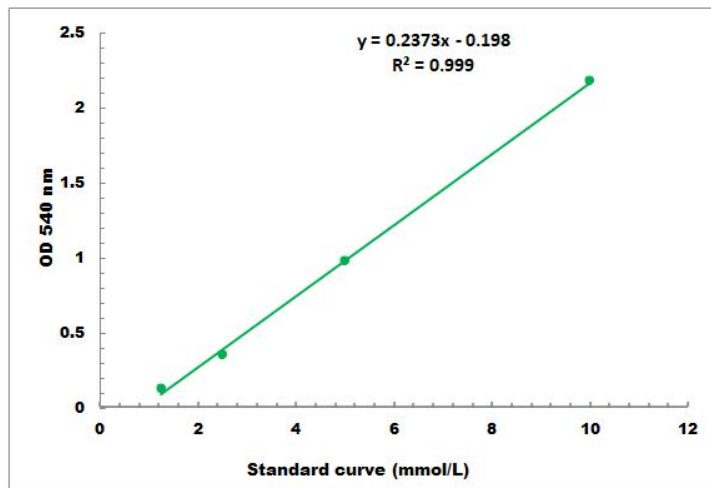
V_{Sample} : the volume of sample, 0.02 ml;

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

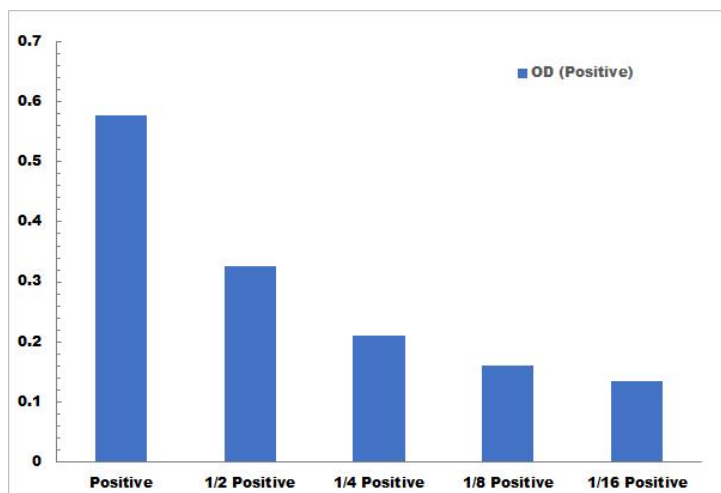
T: the reaction time, 10 minutes.

TYPICAL DATA

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



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



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