

Beta-1, 3-Glucanase
Microplate Assay Kit
Cat #: orb390734 (manual)

Detection and Quantification of Beta-1, 3-Glucanase Activity in Tissue extracts, Cell lysate Samples.

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

INTRODUCTION

β -1, 3-glucanase (EC 3.2.1.73) mainly exists in plant, and it catalyzes the hydrolysis of β -1, 3-glucoside bond. Plant cells would induced to synthesize large amounts of β -1, 3- glucanase when they are infected or in extreme environments. Thus, β -1, 3- glucanase enzyme assays are widely applied in the research of plant pathology and adversity physiology.

β -1, 3-glucanase could hydrolysate laminarin, and cut β -1, 3-glucoside bond to produce reducing terminus. So generating rates of reducing sugar could calculate the activity of enzymes.

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 5 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, mix; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 3 mmol/L.

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 12000g 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 12000g 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 microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	50 μ l	--	--	--
Distilled water	--	50 μ l	--	--
Substrate	50 μ l	50 μ l	--	--
Mix, put it in 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
Mix, put it 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 β -1, 3-glucanase activity is defined as the enzyme liberates 1 μ mol of reducing sugar per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\beta\text{-1, 3-glucanase (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (C_{\text{Protein}} \times \\ &\quad V_{\text{Sample}}) / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\beta\text{-1, 3-glucanase (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times W / \\ &\quad V_{\text{Assay}}) / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W\end{aligned}$$

C_{Standard} : the standard concentration, 3 mmol/L = 3 μ mol/ml;

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

W: the weight of sample, g;

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

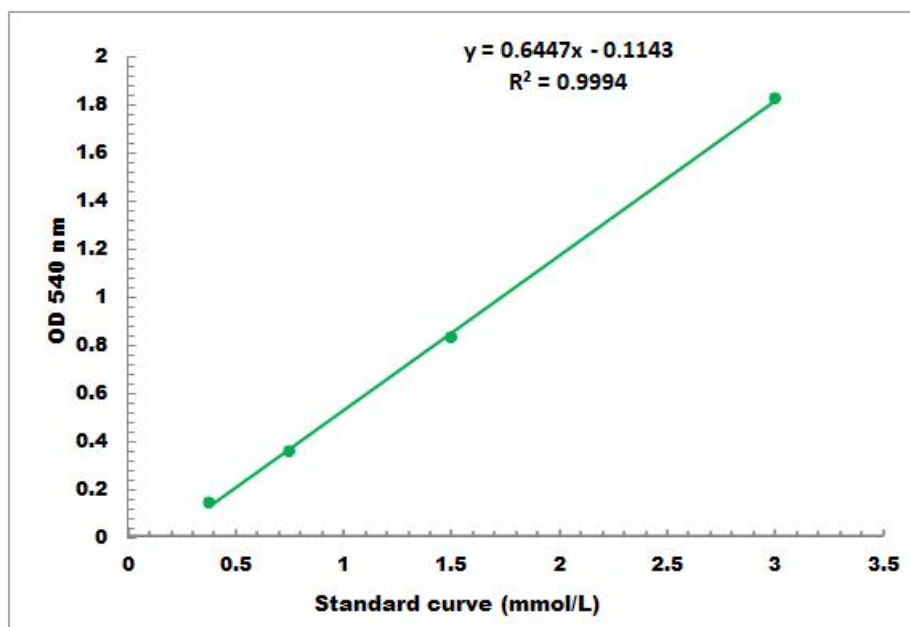
V_{Sample} : the volume of sample, 0.05 ml;

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: 0.3 mmol/L - 3 mmol/L