Beta-1,3-Glucanase Microplate Assay Kit User Manual

Catalog # ASK1028

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.

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

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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 5 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, the concentration will be 0.5 mg/ml.

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 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%, sonication 3s, intervation 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.

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V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	50 µl	--	--	--
Distilled water	--	50 µl	--	--
Substrate	50 µl	50 µl	--	--
Mix, put it in the oven, 37 °C for 30 minutes. Then put it in boiling water for 10 minutes. Add the supernatant into the microplate.				
Supernatant	100 µl	100 µl	--	
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.				

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VI. CALCULATION

Unit Definition: One unit of β-1,3-glucanase activity is the enzyme that generates 1 μg of reducing sugar per minute.

1. According to the protein concentration of sample

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

$$= 33.33 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 33.33 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the protein concentration, 500 μg/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, 30 minutes.

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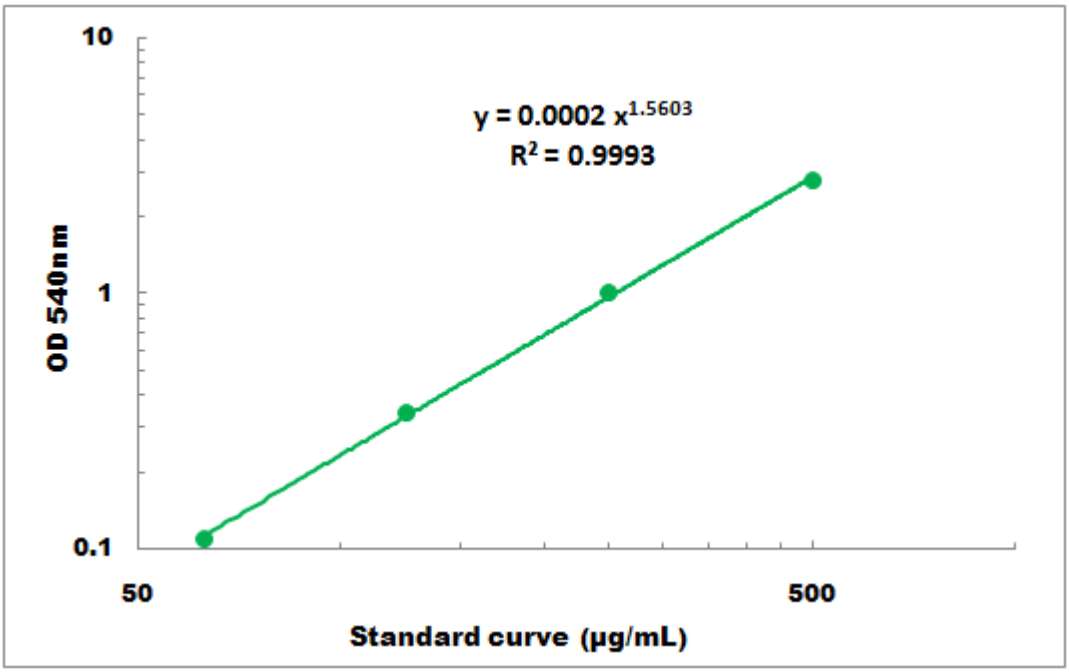
VII. TYPICAL DATA

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



Detection Range: 50 μg/mL - 500 μg/mL

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