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

Catalog # FTA0028

(Version 1.2E)

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.

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.

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, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven

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.

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.				

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.

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;

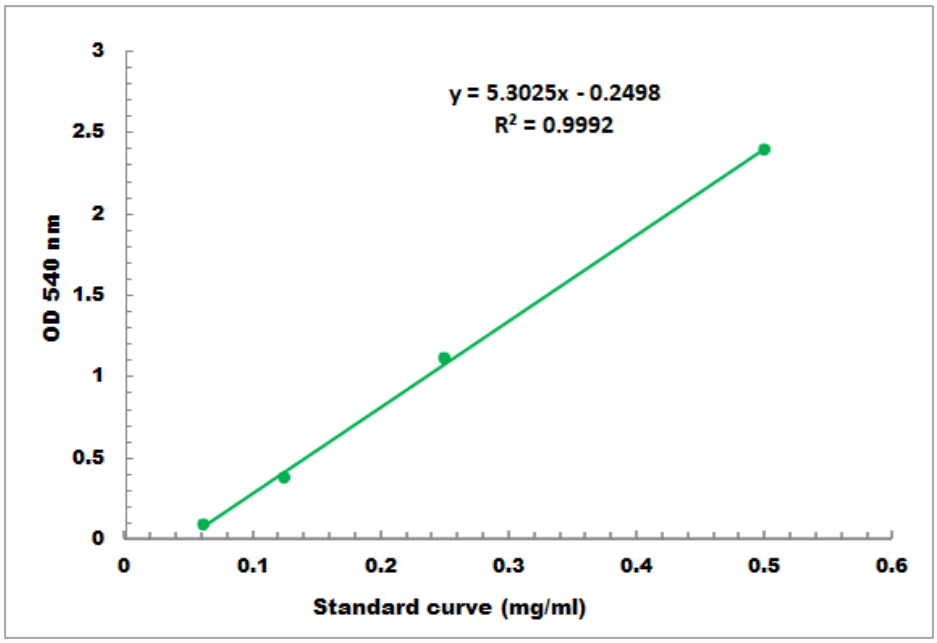
V_{Sample}: the volume of sample, 0.05 ml;

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

T: the reaction time, 30 minutes.

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

VIII. TECHNICAL SUPPORT

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

IX. NOTES