



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

Catalog # CAK1028

(Version 1.3F)

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.

Beta-1,3-Glucanase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring Beta-1,3-Glucanase activity in a variety of samples. 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 mlx 4	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent	10 mlx 1	4 °C
Standard	Powderx 1	4 °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. Hot air circulation oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 10 mmol/L of standard stock solution. Store at 4 °C for 1 month or -20°C for 6-7 months after reconstitution. Then dilute to 3 mmol/L standard top solution by adding 0.3 ml stock solution into 0.7 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.188/0.094/0.047 mmol/L.

Substrate: Add 5 ml distilled water to dissolve before use. Store at 4 °C for 2-3 weeks after reconstitution.

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

V. 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.

VI. 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 10 minutes.				
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, cover the plate adhesive strips, put it into the hot air circulation oven, 90 °C for 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 β -1,3-glucanase activity is defined as the enzyme liberates 1 μ mol of reducing sugar per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

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

C_{Standard} : the concentration of standard, μ mol/mL

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

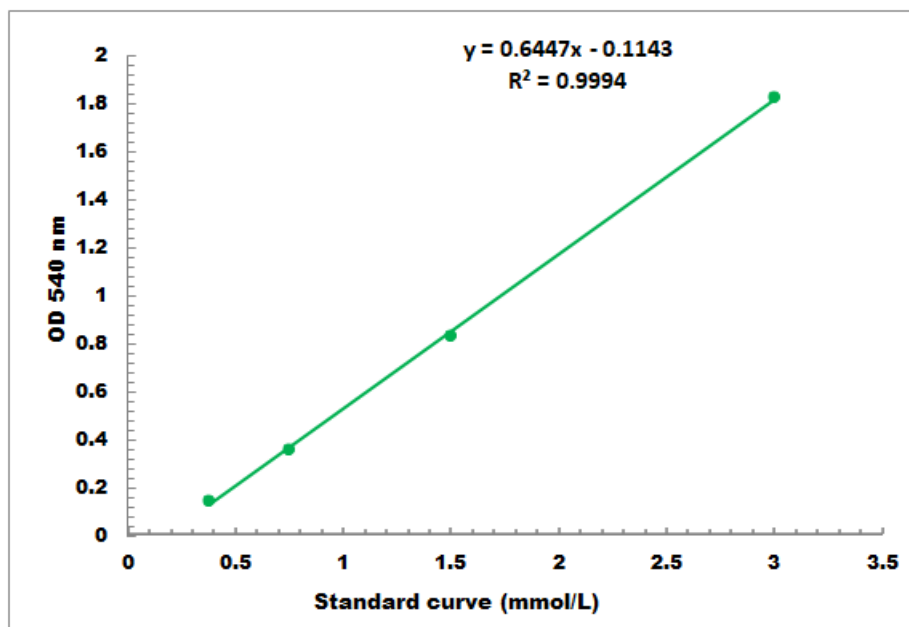
V_{Sample} : the volume of sample in assay procedure, mL

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: 0.3 mmol/L - 3 mmol/L