Chitinase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1088

(Version 1.5F)

Detection and Quantification of Chitinase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Chitinases (EC 3.2.1.14) are hydrolytic enzymes that break down glycosidic bonds in chitin. Chitinase catalyzes the hydrolytic cleavage of the beta-1→4-glycoside bond present in biopolymers of N-acetylglucosamine, primarily in chitin. Chitinases are widely distributed in living organisms and are found in fungi, bacteria, parasites, plants, and animals. They are classified in families based on amino acid sequence similarities.

As chitin is a component of the cell walls of fungi and exoskeletal elements of some animals (including worms and arthropods), chitinases are generally found in organisms that either need to reshape their own chitin or dissolve and digest the chitin of fungi or animals.

Chitinases perform different functions in different organisms. In bacteria, they are mainly involved in nutritional processes. In yeast and various fungi, these enzymes participate in morphogenesis. In animals and plants, chitinases primarily play a role in the defense of the organism against pathogen attack.

Chitinase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring chitinase activity in a variety of samples. The assay is initiated with the enzymatic hydrolysis of the chitin by chitinases. The enzyme catalysed reaction products N-acetylglucosamine react with PDAB, and can be measured at a colorimetric readout at 585 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	5 ml x 1	4 °C
Reaction Buffer	4 ml x 1	RT
Dye Reagent	Powder x 2	4 °C, keep in dark
Dye Reagent Diluent	20 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	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 585 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Boiling water bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 5 mmol/L of standard top solution, store at 4 °C for 1-2 months after reconstitution. 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 5/2.5/1.25/0.625/0.312/0.156/ 0.078 mmol/L.

Reaction Buffer: Heat to 60 °C to dissolve before use.

Dye Reagent: Add 10 ml Dye Reagent Diluent to each bottle to dissolve before use. Keep in dark for immediate use or store at 4 °C for 1 weeks after reconstitution. Heat to dissolve completely if crystals formed.

Positive control: Add 0.2 ml Assay Buffer to dissolve before use. Store at 4 °C for 1-2 weeks or -20 °C for 3 months after reconstitution.

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

V. SAMPLE PREPARATION

1. 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 12,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 12,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For cell culture media

Diluent with same volume of Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tube:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Sample	40 µl	--	--	--	--
Assay Buffer	--	40 µl	--	--	--
Positive Control	--	--	40 µl	--	--
Substrate	40 µl	40 µl	40 µl	--	--
Mix in centrifuge tube, put it in the oven at 37 °C for 1 hour. Centrifuged at 12,000rpm for 10 minutes and transfer the supernatant into a new microcentrifuge tube.					
Supernatant	50 µl	50 µl	50 µl		
Standard	--	--	--	50 µl	--
Distilled water	--	--	--	--	50 µl
Reaction Buffer	25 µl	25 µl	25 µl	25 µl	25 µl
Put it in the boiling water for 4-5 minutes. Cool and transfer the supernatant into the microplate, then add Dye Reagent***.					
Supernatant	50 µl	50 µl	50 µl	50 µl	50 µl
Dye Reagent	150 µl	150 µl	150 µl	150 µl	150 µl
Mix, put it in the oven, 37 °C for 30 minutes, record absorbance measured at 585 nm.					

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.

*** If precipitation was observed after dye reagent addition due to high protein in sample, centrifuged at 12,000 rpm for 10 minutes and transfer the supernatant for following incubation.

VII. CALCULATION

Unit Definition: One unit of Chitinase activity is defined as the enzyme generates 1 μmol of N-acetylglucosamine per minute at 37 °C.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \quad (U/mL)$$

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \quad (U/10^4)$$

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \quad (U/mL)$$

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

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, mmol/L = μmol/ml

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

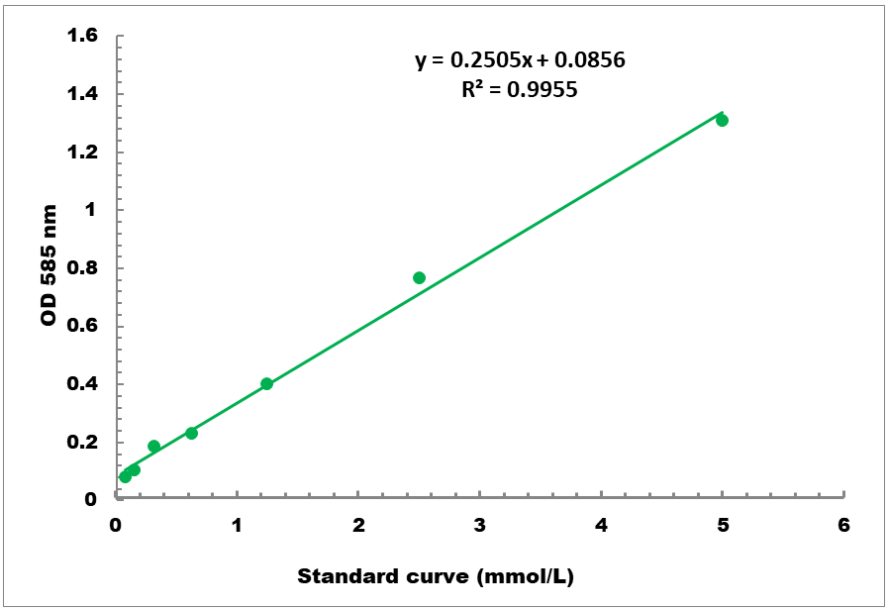
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

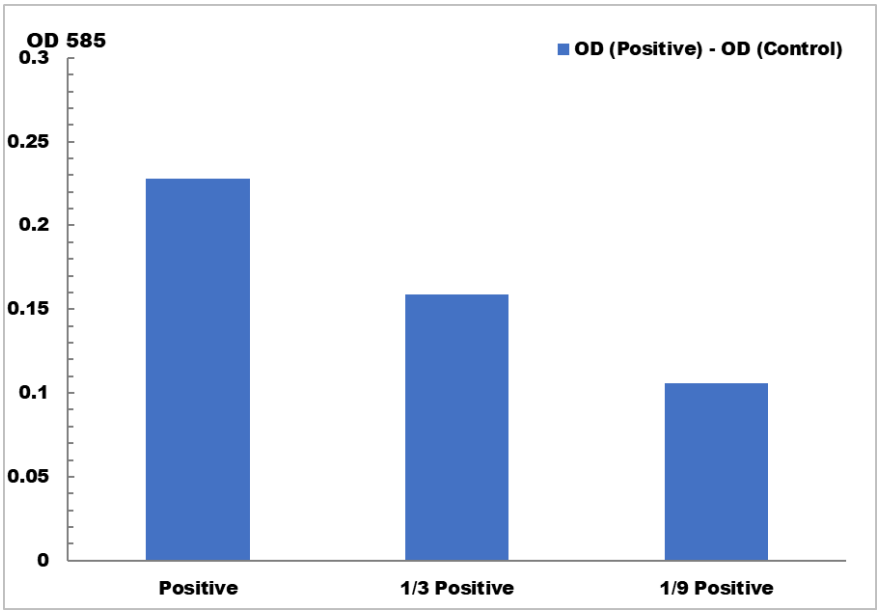
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/L



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