Chitinase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6695

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 .

1. 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 di stributed 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 f ungi, these enzymes participate in morphogenesis. In animals and plants, chitinases primarily play a role in the defense of the organism against pathogen attack.

Chitinase ActivityColorimetric 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.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30mlx 4	4 °C
Substrate	10 mL x 1	4 °C
Reaction Buffer	4mlx 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	12 mL x 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 1 mg/ml of standard top solution , store at 4°C for 1 -2months 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 1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156mg/ml.

Dye Reagent: Add 12 mL Dye Reagent Diluent to dissolve bef ore use.Keep in dark for immediate use or store at 4°C for 2 weeks after reconstitution.

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

5. 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 1 2,000g 4°C for 2 0 minutes , take the supernatant int o 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 2 0 minutes , take the supernatant into a new centrifuge tube and keep it on ice fo r detection.

3. For cell culture media Detect directly.

6. Assay Procedure

Add following reagents in to the micro centrifuge tube :

Reagent*	Sample**	Control	Standard	Blank
Sample	80 µl	--	--	--
Assay Buffer	--	80 µl	--	--
Substrate	80 µl	80 µl	--	--
Mix, put it in the oven, 37°C for 1 hour. Centrifuged at 5000g, 4°C for 10minutes, add80 µlsupernatant into the new microcentrifuge tube.				
Supernatant	80 µl	80 µl	--	--
Standard	--	--	80 µl	--
Distilled water	--	--	--	80 µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Mix, put it in the boiling water for 7 minutes.Centrifuged at 5000g for 2minutesaddthesupernatant into the microplate.				
Supernatant	80 µl	80 µl	80 µl	80 µl
Dye Reagent	120 µl	120 µl	120 µl	120 µl
Mix, put it in the oven, 37°C for 60 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.

7. Calculation

Unit Definition:

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

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

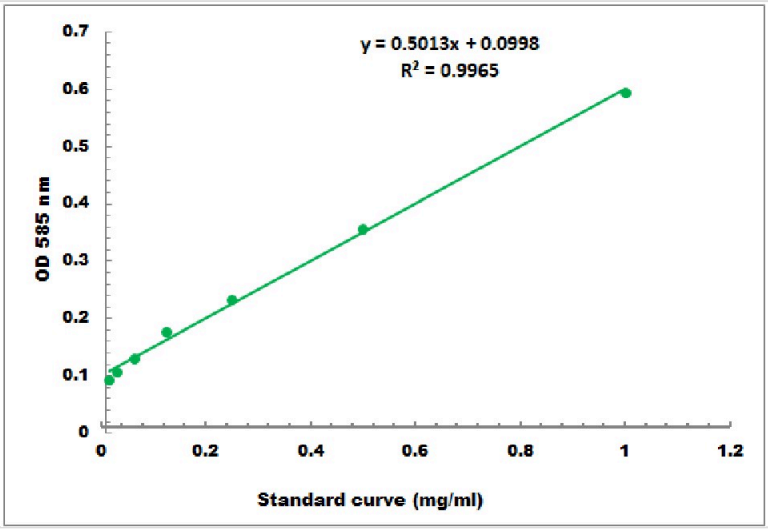
2.4 According to the protein concentration of sample

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

Item	Definition
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, mg/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

8. Typical Data

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



Detection Range : 0.01mg /ml - 1mg /ml