Chitinase Microplate Assay Kit

User Manual

Catalog # CAK1088

(Version 1.3E)

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.

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	10 ml x 1	4 °C
Reaction Buffer	4 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	12 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Dye Reagent: add 12 ml Dye Reagent Diluent to dissolve before use, store at 4 °C.

Standard: add 1 ml distilled water to dissolve before use, mix; the concentration will be 1 mg/ml, store at 4 °C.

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

IV. 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

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge 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 10 minutes, add 80 µl supernatant 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 2 minutes add the supernatant 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:

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$\text{Chitinase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T \times 2$$

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

2. According to the weight of sample

$$\text{Chitinase (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T \times 2$$

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

3. According to the quantity of cells or bacteria

$$\text{Chitinase (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T \times 2$$

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

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 1 mg/ml = 1000 μg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.08 ml;

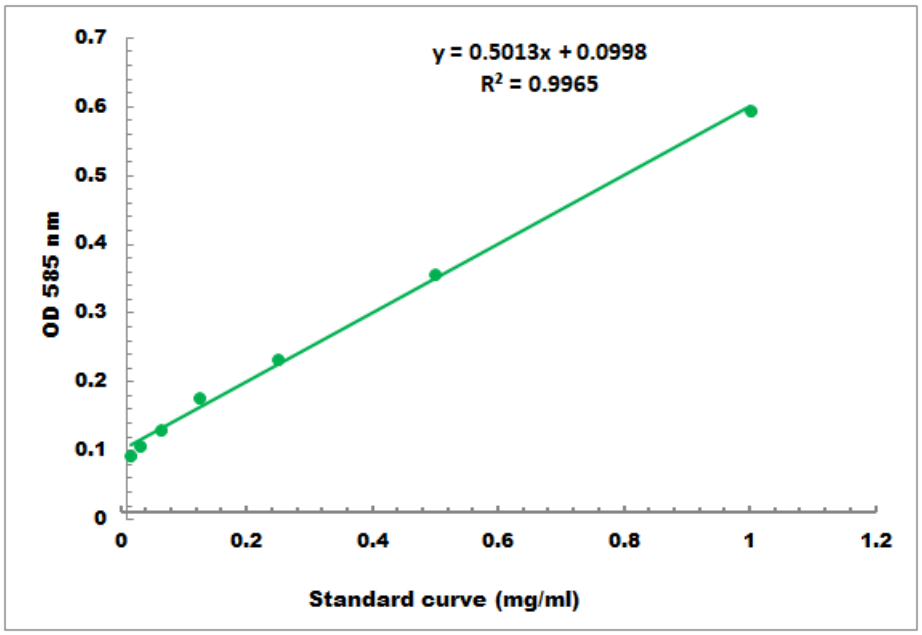
V_{Sample}: the volume of sample, 0.08 ml;

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

T: the reaction time, 60 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.01 mg/ml - 1 mg/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