Chymotrypsin Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1082

(Version 2.1E)

Detection and Quantification ofChymotrypsinActivityin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Chymotrypsin is a digestive enzyme belonging to a super family of enzymes called serine proteases. It uses an active serine residue to perform hydrolysis on the C-terminus of the aromatic amino acids of other proteins. Chymotrypsin is a protease enzyme that cleaves on the C-terminal phenylalanine (F), tryptophan (W), and tyrosine (Y) on peptide chains. It shows specificity for aromatic amino acids because of its hydrophobic pocket.

Chymotrypsin Activity ColorimetricMicroplate Assay Kit is a sensitive assay for determining chymotrypsin activity in various samples. Chymotrypsin activity is determined by the product of paranitroaniline. The reaction products can be measured at a colorimetric readout at 405 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Substrate Diluent	1 ml x 1	4 °C
Activator	Powder x 1	-20 °C
Standard	Powder x 1	4 °C
Standard Diluent	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Enzyme Diluent	3 ml x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolvein 1 mlStandard Diluent to generate 50mmol/Lstock standard solution. Store stock solution at -20 °C for 1 month.Add 0.02 ml stock solution into 0.98 mldistilled water, the concentration will be 1000 µmol/L as top standardsolution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.2/15.6µmol/L.

Substrate Diluent: Warm itto room temperature before use.

Substrate: Briefly centrifuge prior to opening.Add1ml Substrate Diluent before use. Store at -20 °C for 1 month.Prepareready-to-use Substrate Working Solution by mixing Substrate and distilled water at a ratio of 1:9 (v/v).

Activator: Briefly centrifuge prior to opening. Add1 ml Enzyme Diluent to dissolve before use.Store at -80 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add1 ml Enzyme Diluent to dissolve before use.Store at -80 °C for 1 month.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice for 1 hour, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagentsto room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	80 µl	80 µl	80 µl	80 µl	80 µl
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Activator	10 µl	10 µl			10 µl
Distilled water	--	10 µl	20 µl	120 µl	--
Standard	--	--	100 µl	--	--
Substrate	100 µl	100 µl	--	--	100 µl
Mix, incubate at room temperature for 2minutes, record absorbance measured at 405 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.

VII. CALCULATION

Unit Definition: One unit of chymotrypsin activity is defined as the enzyme generates 1 μmolparanitroanilineper minute.

1. According to the slope of the standard curve

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

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

2.1According to the quantity of cells

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

2.2 According to the weight of sample

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

2.2 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

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

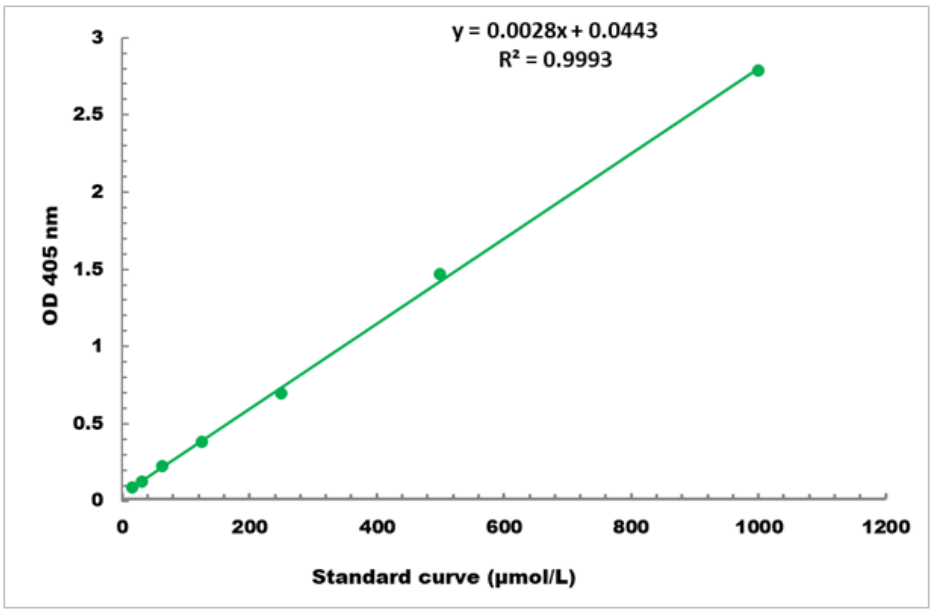
V_{Sample}: the volume of sample in assay procedure, mL

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

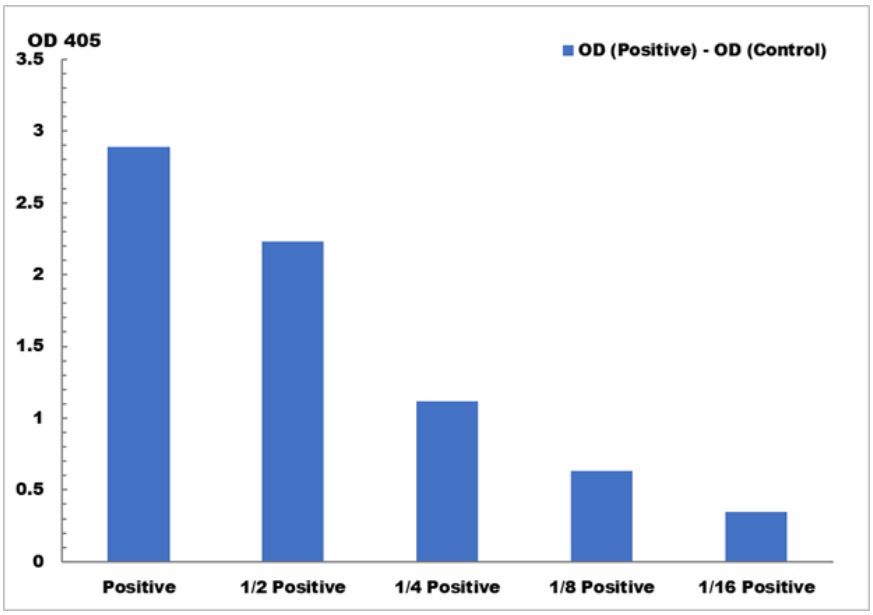
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 10μmol/L - 1000μmol/L



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