Chymotrypsin Microplate Assay Kit

Catalog # AS0082

Detection and Quantification of Chymotrypsin Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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.

The assay is initiated with the enzymatic catalysis of ATEE by Chymotrypsin. The enzyme catalysed reaction products can be measured at a colorimetric readout at 237 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C, keep in dark
Diluent	25 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 19 ml Diluent to dissolve before use.

Standard: add 1 ml Diluent to dissolve before use, the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 237 nm
2. Distilled water
3. 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%, sonication 3s, intervation 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Substrate	190 µl	--	--
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Sample	10 µl	--	--
Mix, measured at 237 nm and record the sample's absorbance of 10th second and 130th second.			

VI. CALCULATION

Unit Definition: One unit of Chymotrypsin activity is defined as the enzyme produce 1 μmol AT in the reaction system per minute.

1. According to the protein concentration of sample

$$\text{Chymotrypsin (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 50 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Chymotrypsin (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 50 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Chymotrypsin (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 50 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$\text{Chymotrypsin (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 50 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 μmol/ml;

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

W: the weight of sample, g;

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

$V_{Standard}$: the volume of standard, 0.2 ml;

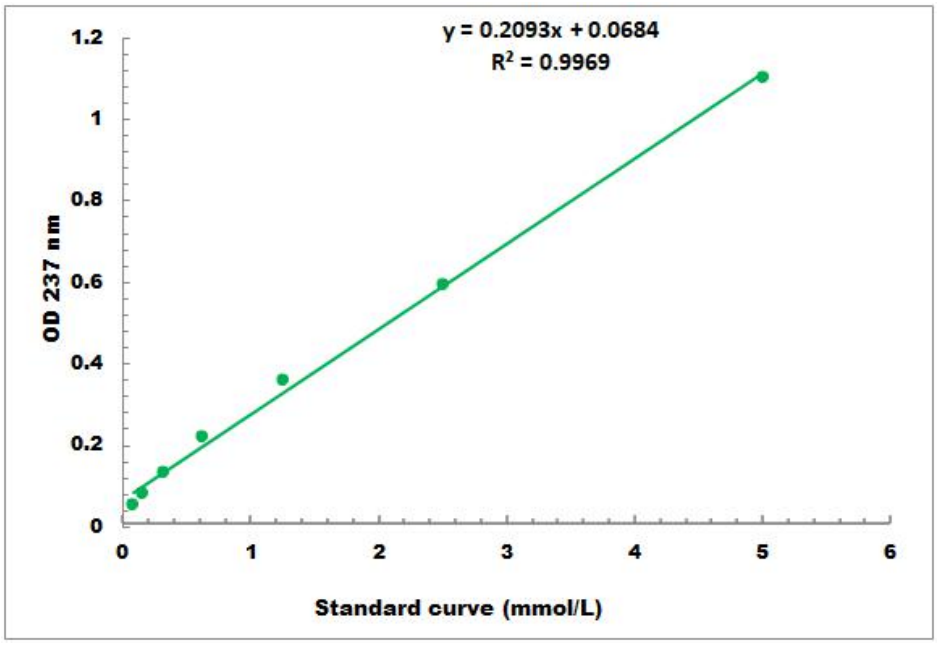
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES