Chymotrypsin Assay Kit

Catalog No.: abx298848

Size: 100 Assays

Storage: Store all components in the dark at 4°C.

Application: For quantitative detection of Chymotrypsin activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine and other biological fluids.

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

Introduction: Chymotrypsin is a digestive enzyme belonging to a superfamily 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.

Abbexa's Chymotrypsin Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Chymotrypsin activity. N-Acetyl-L-Tyrosine Ethyl Ester (ATEE) is hydrolyzed by Chymotrypsin to produce N-Acetyl-L-Tyrosine (AT), which has an absorbance maxima at 237 nm. The intensity of the color is proportional to the reaction product concentration, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Diluent: 25 ml
4. Substrate: 1 vial
5. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (237 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Ethanol
6. Timer
7. Ice
8. Sonicator
9. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 19 ml of Diluent into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of Diluent into the Standard vial and mix thoroughly to prepare the Standard Solution (concentration 5 mmol/L). Ensure that the Standard has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice for 1 hour. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

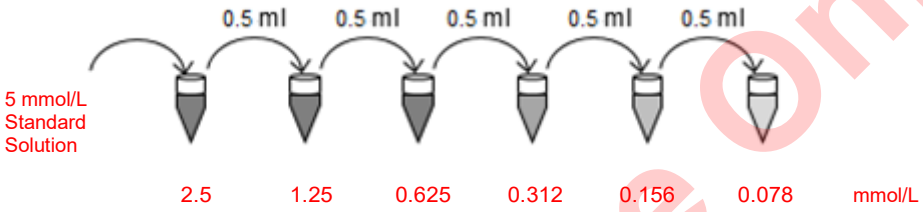
- **Serum and plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 6 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.312 mmol/L, 0.156 mmol/L, and 0.078 mmol/L,. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L Standard Solution to the 1st tube, and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 190 µl of Substrate Solution to the sample wells.
4. Add 200 µl of prepared standard to the standard wells.
5. Add 200 µl of distilled water to the sample wells.
6. Add 10 µl of sample to the sample wells.
7. Tap the plate gently to mix. Start the timer, then read and record absorbance at 237 nm after 10 seconds and after 130 seconds.

C. Calculations

One Unit (U) of Chymotrypsin activity is defined as the quantity of enzyme required to produce 1 µmol of AT per minute.

Chymotrypsin activity per mg of protein:

$$\mathbf{Chymotrypsin\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein} \times T}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{50}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Chymotrypsin activity per g of sample:

$$\mathbf{Chymotrypsin\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{50}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Chymotrypsin concentration per 10^4 cells or bacteria:

$$\mathbf{Chymotrypsin\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{50}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Chymotrypsin activity per ml of serum or plasma:

$$\mathbf{Chymotrypsin\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{50} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$C_{Protein}$	Concentration of protein (in mg/ml)
$C_{Standard}$	Concentration of highest standard (5 mmol/L = 5 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria ($\times 10^4$)
V_{Assay}	Volume of assay buffer (1 ml)
V_{Sample}	Volume of sample (0.01 ml)
$V_{Standard}$	Volume of standard (0.2 ml)
T	Reaction time (2 min)