SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Chymotrypsin Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: AK0234

Size:100T/96S

Components:

Extract solution: 100 mL×1. Storage at 4℃ .

Reagent 1: 10 mL×1. Storage at 4℃ .

Reagent 2: Powder×1. Storage at -20℃, dissolve thoroughly with 1.6 mL methyl alcohol, add to 10 mL with water. Avoid repeated freezing and thawing.

Reagent 3: 5 mL×1. Storage at 4℃ .

Product Description:

Chymotrypsin, also known as chymotrypsin, is a proteolytic enzyme secreted by the pancreas that rapidly decomposes denatured proteins. The function of chymotrypsin is similar to trypsin, but it has the advantages of strong decomposition ability, low toxicity and small adverse reactions. Clinically, chymotrypsin is used for thinning sputum and is effective for both purulent and non-purulent sputum. It is also used for wound healing after trauma or surgery, such as cataract extraction.

Chymotrypsin catalyzes the hydrolysis of BTEE and the product has characteristic light absorption at 256 nm. The chymotrypsin activity was calculated by measuring the rate of increase in light absorption at 256 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro quartz cuvette/ 96 well UV plate, water bath, desk centrifuge, transferpettor, mortar/homogenizer, ice and distilled water.

Sample preparation:

1. Tissue mass (g): Extract solution volume (mL)=1:5- 10. Suggest that add 1 mL Reagent 1 into 0.1 g tissue, fully grinding on ice. Centrifuge at 8000g and 4℃ for 10 min, supernatant (crude enzyme solution) on ice is used for test.
2. Serum can be detected directly.

Procedure:

1. Preheat ultraviolet spectrophotometer/ microplate reader for 30 min, adjust the wavelength to 256 nm, set the counter to zero with distilled water.
2. Keep Reagent 1 at 25℃ water bath for 30 min,
3. Add reagents in 1 mL quartz cuvette as the following:

Reagent name (μL)	Test tube

Reagent 1	90
Reagent 2	90
Reagent 3	20
Sample	20

Mix thoroughly, detect absorbance at 256 nm, A1, detect absorbance at 256 nm after 3 min, A2, ΔA=A2-A1.

Calculation:

A. micro quartz cuvette

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 μmol BTEE in the reaction system per minute at 25℃ every mg protein.

Chymotrypsin (U/mg prot)=(ΔA×Vrv÷ε÷d)÷(Cpr×Vs)÷T=3.8×ΔA ÷Cpr

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 μmol BTEE in the reaction system per minute at 25℃ every g sample.

Chymotrypsin(U/g weight)=(ΔA×Vrv÷ε÷d)÷(W×Vs÷Ve)÷T=3.8×ΔA÷W

(3) Blood:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 μmol BTEE in the reaction system per minute at 25℃ every mL blood.

Chymotrypsin(U/mL)=(ΔA×Vrv÷ε÷d)÷Vs÷T=3.8×ΔA

B. 96 well UV plate

Change d=1 cm to d=0.6 cm.

Vs: Crude enzyme volume (mL), 0.02 mL;

Cpr: Crude enzyme protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

W: Sample weight(g);

Vrv: Total reaction volume,0.22 mL;

Ve: Extraction volume, 1 mL;

T: Reaction time (min), 3 min;

ε: BTEE extinction coefficient, 0.964 mL/μmol/cm;

d: Light path of cuvette, 1 cm.

Note:

1. Dilute sample if ΔA>0. 15 or absorbance value>1; Pay attention to multiplying the dilution ratio in the calculation formula.
2. Concentrate sample or increase sample value if ΔA<0.04, note the calculation formula divided by the concentration times or change the volume.

Experimental example:

1. Take 0. 1g rabbit kidney and add 1ml extract for ice bath homogenization. After centrifugation at 4 ℃ for 10 min, the supernatant was diluted 10 times with the extract, and then the operation was carried out according to the determination steps. measured by micro quartz cuvette. ΔA = A2-A1 = 0.9955-0.9192 = 0.0763.

Chymotrypsin activity (U/g mass) = 3.8×ΔA ÷ W × 10 (dilution ratio) = 28.994 U/g mass.

Related Products：

AK0392/AK0391	Acidic Proteinase(ACP) Activity Assay Kit
AK0390/AK0389	Neutral Proteinase(NP) Activity Assay Kit
AK0386/AK0385	Pepsase Activity Assay Kit