Chymotrypsin Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer

Cat No: BC2330

Size:50T/48S

Components:

Extract solution: 50 mL×1. Storage at 2-8℃.

Reagent I: 25 mL×1. Storage at 2-8℃.

Reagent II: Powder×1. Storage at -20℃. Before use, dissolve reagent II in 4 mL of methanol, and then constant volume to 25 mL with distilled water. Reagent can be stored at -20℃ after dispensing, avoid repeated freezing and thawing..

Reagent III: 5 mL×1. Storage at 2-8℃.

Product Description:

Chymotrypsin, is a protein hydrolase secreted by the pancreas that rapidly breaks down denatured proteins. The function of chymotrypsin is similar to that of trypsin, but chymotrypsin has the advantages of high catabolic capacity, low toxicity and low adverse effects. Chymotrypsin is used clinically for sputum thinning, and is effective in both purulent and non-purulent sputum; chymotrypsin 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 absorption at 256 nm; chymotrypsin activity is calculated by measuring the rate of increase in 256 nm light absorption.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, transferpettor, water bath, 1 mL quartz cuvette, mortar/homogenizer, ice and distilled water.

Sample preparation:

1. Tissue: according to the ratio of tissue mass (g): volume of extraction solution (mL) 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1mL of extraction solution for ice bath homogenization. 8000g, centrifuge at 4℃ for 10min, take the supernatant that is crude enzyme solution.
2. Serum can be detected directly.

Procedure:

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

Reagent name (μL)	Test tube
Reagent I	450
Reagent II	450
Reagent III	100
Sample	100

Add the above reagents to 1mL of quartz cuvette and mix thoroughly.The initial absorbance value A1 was measured at 256 nm, put the cuvette and the react solution to 25℃ water bath for 3 min, take out and dry it quickly, detect absorbance at 256 nm, record as A2, ΔA=A2-A1.

Calculation:

(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 milligram 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 gram sample.

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

(3) Liquid volume:

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 milliliter blood.

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

Vs: Crude enzyme volume, 0.1 mL;

Cpr: Crude enzyme protein concentration, mg/mL; need to detect separately;

W: Sample weight, g;

Vrv: Total reaction volume,1.1 mL;

Ve: Extract solution volume, 1 mL;

T: Reaction time, 3 min;

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

d: Light path of cuvette, 1cm.

Note:

1. It is recommended that the sample be diluted with the extract and then measured if ΔA>0.2 or absorbance value>1, Note the simultaneous modification of the calculation formula;
2. Concentrate sample or increase sample volume if ΔA<0.03, note the calculation formula divided by the concentration times or change the volume.

Experimental example:

1. Take 0.1g rabbit kidney and add 1 mL of extract solution for ice bath homogenization. After centrifugation at 4℃ for 10 min, the supernatant is diluted 10 times with the Extract solution, and then the operation is carried out according to the determination steps. The measured and calculated ΔA = A2-A1 = 1.043-0.94 = 0.103

Chymotrypsin activity (U/g mass) = 3.8 × ΔA ÷ W × 10 (dilution ratio) = 39.14 U/g mass.

Related Products：

BC2280/BC2285	Acidic Proteinase(ACP) Activity Assay Kit
BC2290/BC2295	Neutral Proteinase(NP) Activity Assay Kit
BC2320/BC2325	Pepsase Activity Assay Kit