Chymotrypsin Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer / Microplate Reader

Cat No: BC2335

Size:100T/96S

Components:

Extract solution: 100 mL×1. Storage at 2-8℃.

Reagent I: 10 mL×1. Storage at 2-8℃.

Reagent II: Powder×1. Storage at -20℃. Before use, dissolve reagent II in 1.6 mL of methanol, and then constant volume to 10 mL with distilled water. Reagent can be stored at -20°C 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/microplate reader, micro quartz cuvette/ 96 well UV plate, water bath, desk centrifuge, transferpettor, 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/ microplate reader for 30 min, adjust the wavelength to 256 nm, ultraviolet spectrophotometer set zero with distilled water.
2. Keep Reagent I at 25℃ water bath for 30 min,
3. Add reagents in micro quartz cuvette/ 96 well UV plate as the following:

Reagent name (μL)	Test tube
Reagent I	90
Reagent II	90
Reagent III	20
Sample	20

Add the above reagents to micro quartz cuvette/ 96 well UV plate and mix thoroughly. The initial absorbance value A1 was measured at 256 nm, put the cuvette/ 96 well UV plate and the react solution to 25℃ water bath(microplate reader with temperature control function can adjust the temperature to 25℃) for 3 min, take out and dry it quickly, detect absorbance at 256 nm, record as 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 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 Liquid.

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. 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 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：

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