

Chymotrypsin Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0235

Size:50T/48S

Components:

Extract solution: 50 mL×1. Storage at 4°C .

Reagent 1: 25 mL×1. Storage at 4°C .

Reagent 2: Powder×1. Storage at -20°C, dissolve thoroughly with 4 mL methyl alcohol, add to 25 mL with water. Avoid repeated freezing and thawing.

Reagent 3: 5 mL×1. Storage at 4°C .

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, desk centrifuge, transferpettor, water bath, 1 mL quartz cuvette, 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 8000 g and 4°C for 10 min, supernatant (crude enzyme solution) on ice is used for test.
2. Serum can be detected directly.

Procedure:

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

Reagent name (μL)	Test tube
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Reagent 1	450
Reagent 2	450
Reagent 3	100
Sample	100

Mix thoroughly, detect absorbance at 256 nm, A1, put the cuvette and the react solution to 25°C water bath for 3 min, take out and dry it quickly, detect absorbance at 256 nm, A2, $\Delta A = A2 - A1$.

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°C every mg protein.

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$$\text{Chymotrypsin (U/mg prot)} = (\Delta A \times V_{rv} \div \epsilon \div d) \div (C_{pr} \times V_s) \div T = 3.8 \times \Delta A \div C_{pr}$$

(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°C every g sample.

$$\text{Chymotrypsin (U/g weight)} = (\Delta A \times V_{rv} \div \epsilon \div d) \div (W \times V_s \div V_e) \div T = 3.8 \times \Delta A \div W$$

(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°C every mL blood.

$$\text{Chymotrypsin (U/mL)} = (\Delta A \times V_{rv} \div \epsilon \div d) \div V_s \div T = 3.8 \times \Delta A$$

V_s : Crude enzyme volume (mL), 0.1 mL;

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

W : Sample weight(g);

V_{rv} : Total reaction volume, 1.1 mL;

V_e : Extract solution volume, 1 mL;

T : Reaction time (min), 3 min;

ϵ : BTEE extinction coefficient, 0.964 mL/ $\mu\text{mol}/\text{cm}$;

d : Light path of cuvette, 1cm.

Note:

1. Dilute sample if $\Delta A > 0.2$ or absorbance value > 1 ;
2. Concentrate sample or increase sample volume if $\Delta A < 0.03$, 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°C 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 $\Delta A = A_2 - A_1 = 1.043 - 0.94 = 0.103$

Chymotrypsin activity (U/g mass) = $3.8 \times \Delta A \div W \times 10$ (dilution ratio) = 39.14 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