Trypsase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0704

Size: 50T/48S

Components:

Extraction reagent: 50 mL×1 bottle. Storage at 4℃.

Reagent II: powder×1 bottle. Storage at 4℃, dissolve with 1 mL distilled water before use.

Reagent III: 50 mL×1 bottle. Storage at 4℃.

Product Description:

Trypsin is a serine protease from the PA clan superfamily, found in the digestive system of many vertebrates, where it hydrolyses proteins. In the duodenum, trypsin catalyzes the hydrolysis of peptide bonds, breaking down proteins into smaller peptides. The peptide products are then further hydrolyzed into amino acids via other proteases, rendering them available for absorption into the blood stream. Trypsin is widely used in the treatment of local edema, hematoma and abscess due to the pyothorax, hemothorax, surgical inflammation, ulcer, traumatic injury, etc. Trypsin catalyzes and hydrolyzes the ester bonds of BAEE, producing BA, BA has absorpotion at 253 nm. The activity of trypsin is calculated by measuring the increase rate of 253 nm absorbance.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, centrifuge, transferpettor, 1 mL quartz cuvette, water-bath, mortar/ homogenizer, ice and distilled water.

Procedure

I. Sample extraction

Tissue sample：add 0.1 g tissue to 1 mL Extraction reagent, homogenate in ice bath. Centrifuge at 10000 rpm for 10 min at 4℃ and get the supernatant solution, set on ice to be tested.

Enzyme sample：take 1 mg enzyme powder，add 1 mL Extraction reagent，mix thoroughly before testing on ice (gradient dilution is recommended to ensure the accuracy of the experiment).

II. Detection

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 253 nm and set zero with distilled water.
2. Prepare working solution：Accordance Reagent I: Reagent II=2:97, preheat working solution at 37℃ water bath for 30 min. Prepare the solution according to your need.
3. Blank tube (ΔA_B): Add 990 μL working solution and 10 μL distilled water to 1 mL glass cuvette, mix thoroughly, detect the 253 nm absorbance at 0s (A1) and 60s (A2), record ΔA_B=A2-A1.

4. Test tube (ΔA_T): Add 990 μL working solution and 10 μL sample extractionto 1 mL quartz cuvette, mix thoroughly, detect the 253 nm absorbance at 0s (A3) and 60s (A4), record ΔA_T=A4-A3.

III. Calculation：

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the producing 0.001 absorbance change in 253 nm in 1 mL reaction system per minute at 37℃ every mg protein.

Trypsin (U/mg prot)=$(\Delta A_T-\Delta A_B) \div 0.001 \div (Cpr \times V1) \div T = 100000 \times (\Delta A_T-\Delta A_B) \div Cpr$

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the producing 0.001 absorbance change in 253 nm in 1 mL reaction system per minute at 37℃ every g sample.

Trypsin (U/mg weight)= $(\Delta A_T-\Delta A_B) \div 0.001 \div (W \times V1 \div V2) \div T = 100000 \times (\Delta A_T-\Delta A_B) \div W$

W: Tissue weight(g);

V1: Enzyme volume (mL) in reaction system, 10 μL =0.01 mL;

V2:Total Enzyme extraction volume(mL), 1 mL;

Cpr:The protein concentration of enzyme solution(mg/mL);

T: Reaction time (min), 1 min.

Note:

1. Take 1~2 different samples for prediction before test and ensure the absorbance range in 0.01-0.15.
2. If the measured result ΔA is negative, the reagent can be diluted (2 times or 4 times) and then test it.

Experimental example:

1. Take 0.1g pancreas and add 1ml extract for ice bath homogenization, centrifugation at 4℃ for 10 minutes at 10000 rpm, take the supernatant, put it on ice, operate according to the determination steps, and calculate ΔA_T = A4-A3 = 0.546-0.524 = 0.022, ΔA_B = A2-A1 = 0.464-0.464 = 0

Trypsin (U/g mass) = $10^5 \times (\Delta AT - \Delta AB) \div W$ = 22000 U/g mass

Recent Product Citations：

[1] Ren Zhang, Ruolun Wei, Wei Du, et al. Long noncoding RNA ENST00000413528 sponges microRNA-593-5p to modulate human glioma growth via polo-like kinase 1. CNS Neuroscience & Therapeutics. March 2019;(IF4.458)

Related Products：

NA0707/NA0466	Acidic Proteinase(ACP) Activity Assay Kit
NA0706/NA0465	Neutral Proteinase(NP) Activity Assay Kit
NA0703/NA0462	Pepsase Activity Assay Kit
NA0702/NA0461	Chymotrypsin Activity Assay Kit