Trypsase Activity Assay Kit

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

Operation Equipment: Microplate Reader/Spectrophotometer

Catalog Number: AK0387

Size:100T/96S

Components:

Reagent I: 100 mL×1 bottle. Storage at 4℃ .

Reagent Ⅱ: Powder×1 bottle. Storage at 4°C, dissolve with 0.5 mL of distilled water before use.

Reagent Ⅲ: 20 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:

Microplate reader/ spectrophotometer， transferpettor, micro quartz cuvette/96 well UV plate, centrifuge, water-bath, transferpettor, mortar/ homogenizer, ice, 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°C and get the supernatant solution, set on ice to be tested.

Enzymesample： 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 or microplate reader for 30 minutes, adjust wavelength to 253 nm, set zero with distilled water.
2. Prepare working solution: Accordance Reagent I: Reagent II=2:97, preheat working solution at 37°C water bath for 30 min. Prepare the solution according to your need.
3. Blank tube (ΔA_B): Add 198 μL working solution and 2 μL distilled water to micro quartz cuvette or 96 well UV plate, mix thoroughly and detect the 253 nm absorbance at 0s(A1) and 60s(A2), record ΔA_B=A2-

A1.

4. Test tube (ΔA_T): Add 198 μL working solution and 2 μL enzyme solution to micro quartz cuvette or 96 well UV plate, mix thoroughly and detect the 253 nm absorbance at 0s(A3) and 60s(A4), record ΔA_T=A4-A3.

III. Calculation

A. Calculation formula of determination with micro quartz cuvette

1.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)= $(\Delta A_T-\Delta A_B)\div0.001\div(Cpr\times V1)\div T\times(V3\div V4)=100000\times(\Delta A_T-\Delta A_B)\div Cpr$

2.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)= $(\Delta A_T-\Delta A_B)\div0.001\div(W\times V1\div V2)\div T\times(V3\div V4)=100000\times(\Delta A_T-\Delta A_B)\div W$

Cpr: Sample concentration (mg/mL).

W: Tissue weight (g).

V1: Enzyme solution volume, 2 μL=0.002 mL.

V2:Total sample volume, 1 mL

T: Reaction time, 1 min.

V3: Total reaction volume, 198 μL+2 μL=200 μL=0.2 mL.

V4: 1 mL reaction system volume.

B. Calculation formula of determination with micro quartz cuvette

1.Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the producing 0.0005 absorbance change in 253 nm in 1 mL reaction system per minute at 37℃ every mg protein.

Trypsin (U/mg prot)= $(\Delta A_T-\Delta A_B)\div0.001\div(Cpr\times V1)\div T\times(V3\div V4)=2\times100000\times(\Delta A_T-\Delta A_B)\div Cpr$

2.Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the producing 0.0005 absorbance change in 253 nm in 1 mL reaction system per minute at 37℃ every g sample.

Trypsin (U/mg weight)= $(\Delta A_T-\Delta A_B)\div0.001\div(W\times V1\div V2)\div T\times(V3\div V4)= 2\times100000\times(\Delta A_T-\Delta A_B)\div W$

Cpr: Sample concentration (mg/mL).

W: Tissue weight (g).

V1: Enzyme solution volume, 2 μL=0.002 mL.

V2:Total sample volume, 1 mL

T: Reaction time, 1 min.

V3: Total reaction volume, 198 μL+2 μL=200 μL=0.2 mL.

V4: 1 mL reaction system volume.

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Note:

1. Take 1~2 samples for prediction before test and ensure the absorbance range in 0.01-0. 15. (If 96 well UV plate is used, the variation range is 0.01-0.08).

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 1.25 mg/mL trypsin solution, operate according to the determination steps, use 96 well UV plate to calculate ΔA_T = A4-A3 = 0.363-0.344 = 0.019, ΔA_B =A2-A1 = 0.325-0.324 = 0.001

Trypsin (U/mg prot) = $2\times10^5 \times(\Delta A_T - \Delta A_B) \div Cpr$ = 2880 U/mg prot.

Recent Products 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：

AK0392/AK0391	Acidic Proteinase(ACP) Activity Assay Kit
AK0390/AK0389	Neutral Proteinase(NP) Activity Assay Kit
AK0386/AK0385	Pepsase Activity Assay Kit
AK0235/AK0234	Chymotrypsin Activity Assay Kit