Pepsase Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer /Microplate Reader

Cat No: BC2325

Size:100T/48S

Components:

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

Reagent I: Powder×1. Storage at 2-8℃. Add 10 mL of reagent II dissolve fully before use.

Reagent II: 15 mL×1. Storage at 2-8℃.

Reagent III: Powder×1. Storage at 2-8℃. Add 10 mL of distilled water dissolve fully before use.

Product Description:

Pepsin is secreted by major cells of the gastric mucosa which break down proteins in food into small peptides. It is generally used for the identification of low-acid nerve disease. chronic gastritis, chronic gastric dilatation, chronic duodenitis can also cause a decrease in pepsin secretion.

Pepsin can catalyze the hydrolysis of hemoglobin form tyrosine, which has characteristic absorbance at 275 nm. The enzyme activity can be calculated by measuring the change of the absorbance.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer /microplate reader, micro quartz cuvette/96 well UV plate, desk centrifuge, water bath, adjustable transferpettor, mortar/homogenizer, ice and distilled water.

Sample preparation:

Add 1 mL extract solution into 0.1 g tissue or add 0.1 mL gastric juice to 0.9 mL extract solution, fully grinding on ice. Centrifuge at 10000 rpm and 4℃ for 10 min. Supernatant (crude enzyme solution) on ice is used for test.

Procedure:

1. Preheat ultraviolet spectrophotometer /microplate reader for 30 min, adjust the wavelength to 275 nm, ultraviolet spectrophotometer set zero with distilled water.
2. Add the following reagents:

Reagent name (μL)	Test tube (T)	Contract tube (C)
Sample	20	-
Reagent I	100	100
Mix thoroughly, keep in 37℃ for 10 min.		
Reagent III	100	100

Mix thoroughly for 1 min.		
Sample (μL)	-	20
Mix thoroughly, centrifuge at 10000 rpm and 4℃ for 10 min, take supernatant in micro quartz cuvette/96 well UV plate, detect absorbance at 275 nm, $\Delta A=\Delta A_T-\Delta A_C$.		

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 hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every milligram protein.

Pepsase(U/mg prot)= (ΔA÷ε÷d×Vrv)÷(Vs×Cpr)÷T =0.786×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every gram sample.

Pepsase (U/g weight)= (ΔA÷ε÷d×Vrv)÷(Vs÷Vsv×W)÷T=0.786×ΔA÷W

3. Liquid volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every milliliter liquid.

Pepsase(U/mL)= (ΔA÷ε÷d×Vrv)÷(Vs÷Vsv×Vl)÷T=7.86×ΔA

Cpr: Sample protein concentration (mg/mL); need to detect separately;

Vrv: total reaction volume, 0.22 mL;

Vsv: crude enzyme volume, 1 mL;

T: reaction time, 10 min;

Vs: sample volume, 0.02 mL;

Vl: liquid volume, 0.1 mL;

ε: tyrosine molar extinction coefficient, 1.4 mL/μmol/cm;

d: light path of cuvette, 1 cm;

W: sample weight(g).

B. 96 well UV plate:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every milligram protein.

Pepsase(U/mg prot)= (ΔA÷ε÷d×Vrv)÷(Vs×Cpr)÷T =1.31×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every gram sample.

Pepsase (U/g weight)= (ΔA÷ε÷d×Vrv)÷(Vs÷Vsv×W)÷T=1.31×ΔA÷W

3. Liquid volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of

hemoglobin to 1 μmol of tyrosine in the reaction system per minute at 37℃ every milliliter liquid.

Pepsase(U/mL)= (ΔA÷ε÷d×Vrv)÷(Vs÷Vsv×Vl)÷T=13.1×ΔA

Cpr: Sample protein concentration (mg/mL); need to detect separately;

Vrv: total reaction volume, 0.22 mL;

Vsv: crude enzyme volume, 1 mL;

T: reaction time, 10 min;

Vs: sample volume, 0.02 mL;

Vl: liquid volume, 0.1 mL;

ε: tyrosine molar extinction coefficient, 1.4 mL/μmol/cm;

d: light path of cuvette, 0.6 cm;

W: sample weight(g).

Experimental example:

1. Take 0.1g mouse stomach, add 1 mL of extract solution, grind it thoroughly, centrifuge it at 10000rpm and 4℃ for 10 minutes, dilute the supernatant twice, place it on ice, operate according to the determination steps, measure with micro quartz cuvette and calculate $\Delta A = A_T - A_C$= 1.3345-1.2195 = 0.115, calculate the enzyme activity according to the sample mass

Pepsin activity (U/g mass) = 0.786 ×ΔA ÷ W × 2 (dilution ratio) = 1.808 U/g mass.

Related Products:

BC2280/BC2285 Acidic Proteinase(ACP) Activity Assay Kit

BC2290/BC2295 Neutral Proteinase(NP) Activity Assay Kit

BC2330/BC2335 Chymotrypsin Activity Assay Kit