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Pepsase Activity Assay Kit

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

Operation Equipment: Spectrophotometer /Microplate Reader

Cat No: AK0385

Size:100T/48S

Components:

Extract solution: 50 mL×1. Storage at 4℃ .

Reagent 1:Powder×1. Storage at 4℃ and protect from light, dissolve thoroughly with 10 mL Reagent 2 before use.

Reagent 2: 15 mL×1. Storage at 4℃ .

Reagent 3:Powder×1. Storage at 4℃, dissolve thoroughly with 10 mL distilled water 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 catalyzes 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:

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 spectrophotometer/microplate reader for 30 min, adjust the wavelength to 275 nm, set the counter to zero with distilled water.

2. Add the following reagents:

	Test tube (T)	Contract tube (C)
Sample (μL)	20	-
Reagent 1 (μL)	100	100
Mix thoroughly, keep in 37℃ for 10 min.		
Reagent 3 (μL)	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, ΔA=ΔA(T) -ΔA(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 mg 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 g 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 mL 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, suggest use PC0020, BCA Protein Assay Kit;

Vrv: total reaction volume, 0.22 mL;

Vsv: crude enzyme volume, 1 mL;

T: reaction time (min), 10 min;

Vs: sample volume (mL), 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 mg 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 g 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 mL 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, suggest use PC0020, BCA Protein Assay Kit;

Vrv: total reaction volume, 0.22 mL;

Vsv: crude enzyme volume, 1 mL;

T: reaction time (min), 10 min;

Vs: sample volume (mL), 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：

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