Pepsin Assay Kit

(Cat/No:BC038 Size:50T/24S)

1. Principle of Measurement

Pepsin catalyzes the hydrolysis of protein and the product of Tyrosine can reduce the phenol reagent to blue compound and thus the measurement of absorbance can be used to calculate the pepsin activity.

2. Composition and Preparation (The kit is valid for 1 month)

Reagent I: 1 Bottle×24ml. Preserved at 4°C for 3 months.
Reagent II: 2 Bottles×6ml. Preserved at 4°C for a month.
Reagent III: 1 Bottle×100ml. Preserved at 4°C for 3 months.
Reagent IV: 2 Bottles×9ml. Preserved without light struck at 4°C for 2 months.
Reagent V: Tyrosine stock solution with conc. 1mg/ml. 1 Bottle×1ml.
Diluent 1 Bottle×20ml. Both should be preserved at 4°C for a month.
Preparation of 50μg/ml standard solution: Dilute 50μl stock solution with diluent to the final volume of 1 ml. The solution can be preserved at 4°C for 10 days.
Reagent VI: Homogeneous medium. 2 Bottle×60ml. Preserved at 4°C for a month.

3. Procedures

I. Pre-treatment

Gastric acid sample: Pre-measurement can be done first and dilute the supernatant with reagent VI were the results too high.
Tissue sample: Weigh the tissue precisely, and add physiological saline with the ratio 1g tissue to 9ml saline. Homogenize the mixture to 10% homogenate and centrifuge the homogenate at 2,500 rpm for 10 min. Pre-test the 10% homogenate supernatant and were the results too high, dilute the supernatant with reagent VI with proper ratio.

II. Regulate the sample and Reagent to RT before the measurement

Place at RT if the local temperature exceeds 20°C or warm the reagents at 37°C for 10 min were the local temperature below 20°C.

III. Procedures

Enzymatic Reaction

Compositions (ml)	Sample	Reference
Pre-treated Supernatant	0.04	0.04

Warm at 37°C in water bath for 2 min		
Reagent II	0.20	0.20
	Mix well, and conduct the reaction at 37℃ for exactly 10 minutes.	Mix well and then add reagent I **immediately**.
Reagent I	0.40	0.40

Mix thoroughly and warm at 37°C for 10 min. Centrifuge at 3,500 rpm for 10 min and extract 0.3 ml supernatant for further reaction.

Chromogenic Reaction

Compositions (ml)	Blank	Standard	Sample	Reference
Reagent V Diluent	0.30			
Reagent V Standard		0.30		
Supernatant			0.30	0.30
Reagent III	1.50	1.50	1.50	1.50
Reagent IV	0.30	0.30	0.30	0.30

Mix thoroughly and warm the mixture and 37°C for 20 min.

Regulate the spectrophotometer with distilled water and measure the optical density (OD) absorbed by each tube at 660 nm with 1cm path length.

Note: For mass number of samples, only 1-2 samples for standard and blank measurement respectively. For each sample, one corresponding reference should be done.

4. Calculation Formula and Examples

I. Gastric Acid Sample

a. Definition: One enzyme activity unit is defined as 1μg Tyrosine generated with protein hydrolysis catalyzed by enzymes within 1ml gastric acid.

b. Formula

$$Enzyme\ Activity\ U/ml = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \times \frac{CoD}{Pretreatment}$$

Note: CoD represents the coefficient of dilution.

c. Example

Rat gastric acid was diluted with reagent VI with 1:1 ratio and then measured as mentioned above. The OD values were 0.039, 0.546, 0.213 and 0.069 respectively.

$$\underset{U/ml}{Enzyme\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \times \underset{Pretreatment}{CoD}$$

$$= \frac{0.213 - 0.069}{0.546 - 0.039} \times 50 \div 10 \times \frac{0.64}{0.04} \times 2 = 45.4U/ml$$

II. Tissue Samples

a. Definition: One enzyme activity unit is defined as 1μg Tyrosine generated with protein hydrolysis catalyzed by enzymes within 1g protein.

b. Formula

$$\underset{U/mg}{Enzyme\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \div \frac{C_{Protein}}{mg/ml}$$

c. Example

i. 10% homogenate was prepared with razor clam gastric tissues and measured. The OD values were 0.039, 0.546, 0.208 and 0.066 respectively. Also, the protein concentration of 10% homogenate was 1.45mg/ml.

$$\underset{U/mg}{Enzyme\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{0.208 - 0.066}{0.546 - 0.039} \times 50 \div 10 \times \frac{0.64}{0.04} \div 1.45 = 15.5U/mg$$

ii. 10% homogenate was prepared with clam gastric tissues and measured. The OD values were 0.039, 0.546, 0.128 and 0.047 respectively. Also, the concentration of the homogenate was measured to be 1.14g/ml.

$$\underset{U/mg}{Enzyme\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{0.128 - 0.047}{0.546 - 0.039} \times 50 \div 10 \times \frac{0.64}{0.04} \div 1.14 = 11.2U/mg$$

iii. 5% homogenate was prepared with fish digestive tissues and measured with procedures mentioned above. The OD values were 0.039, 0.546, 0.351 and 0.205 respectively. The protein concentration of 5% homogenate was 1.576mg/ml.

$$\underset{U/mg}{Enzyme\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{standard}}{50\mu g/ml} \div \frac{T}{10\ min} \times \frac{V_{Total}(0.64ml)}{V_{sample}(0.04ml)} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{0.351 - 0.205}{0.546 - 0.039} \times 50 \div 10 \times \frac{0.64}{0.04} \div 1.576 = 14.6U/mg$$

Appendix I Standard Curve Establishment

1. Procedures

Tube No.	0	1	2	3	4	5
Reagent V Diluent	0.3	0.24	0.18	0.12	0.06	
0.1mg/ml Tyrosine Standard Solution		0.06	0.12	0.18	0.24	0.3
Reagent III	1.5	1.5	1.5	1.5	1.5	1.5
Reagent IV	0.3	0.3	0.3	0.3	0.3	0.3

Mix thoroughly and warm the mixture and 37°C for 20 min.

Regulate the spectrophotometer with distilled water and measure the optical density (OD) absorbed by each tube at 660 nm with 1cm path length.

2. Results

Tube No.	0	1	2	3	4	5
Tyrosine Conc.（μg/ml）	0	20	40	60	80	100
OD measured	0.016	0.217	0.410	0.607	0.800	0.993
$\Delta OD(OD_{Sample}- OD_{Reference})$	0.000	0.201	0.394	0.591	0.784	0.977

3. Standard Curve