Trypsin Assay Kit

(Cat.No.:BC039 Size:50T/48S,25T/24S)

1. Significance of Measurement

I. Increase: Most patients suffering acute pancreatitis or chronic kidney diseases may have significant increases in trypsin activity. Half of the patients suffering pancreatic cancer or chronic pancreatitis would have higher trypsin activities. And around 20% patients who are not suffering the abdominal pain but pancreatic abnormal like cholecystitis or peptic ulcer are possibly with higher trypsin activity.

II. Decrease: Mostly found from patients suffering chronic pancreatitis, biliary diseases or chronic cholecystitis.

2. Principle (Ultraviolet colorimetry)

Trypsin catalyzes the hydrolysis of arginine ethyl ester and thus the absorbance of the solution would increase at 253 nm. Measure such OD difference and the enzyme activity can be calculated.

3. Composition and Preparation (The kit is valid for 6 months)

I. For 50T/ 48 Samples

Reagent I: Substrates for Trypsin: 3 Bottles of Powder.

Diluent: 3 Bottles×30ml. Both can be preserved at 2-8°C for 6 months.

Preparation of Reagent I Solution: Dissolve one bottle of powder with one bottle of diluent thoroughly. The solution should be prepared right before use and can be preserved at 2-8°C for 24 hours.

Reagent II: Homogenate Medium for Sample Pre-Treatment: 2 Bottles×60ml.

Preserved at 2-8°C for 6 months.

II. For 25T/ 24 Samples

Reagent I: Substrates for Trypsin: 2 Bottles of Powder.

Diluent: 2 Bottles×30ml. Both can be preserved at 2-8°C for 6 months.

Preparation of Reagent I Solution: Dissolve one bottle of powder with one bottle of diluent thoroughly. The solution should be prepared right before use and can be preserved at 2-8°C for 24 hours.

Reagent II: Homogenate Medium for Sample Pre-Treatment: 1 Bottle×60ml.

Preserved at 2-8°C for 6 months.

4. Procedures

I. Serum Samples

a. Pretreatment: The serum can be measured directly or dilute with reagent II in order to get desirable absorbance value.

b. Procedures

Compositions (ml)	Blank	Sample
Reagent I Solution	1.5	1.5
	Pre-warm at 37°C for 5 min.	
Sample		a
Reagent II Solution	a	

Record the time after the addition of the sample. Mix the solution and for each type of solution, transfer it into a cuvette with 0.5cm path length. Record the absorbance value A_1 30 seconds after the addition of the sample. Place the cuvette into a thermostatted compartment at 37°C. Record the absorbance value A_2 at the timing of 20 minutes 30 seconds.

Note: It is recommended to do the pre-test before and under the circumstance of A_2-A_1 out of the range of 0.05-0.8, the sample should be diluted or concentrated (increase the sample amount) to receive results within the range permitted.

c. Detailed Procedures

i. Regulate both two cuvettes with double distilled water (DDW) spectrophotometrically at 253 nm. (One cuvette for blank and the other one for sample measurement).

ii. Warm the reagent I solution at 37°C for more than 5 min.

iii. Add 1.5ml pre-warmed reagent I into both tubes and then weigh a ml (a is an arbitrary number) sample serum or reagent II solution into the corresponding tube. Start timing and shake the tube quickly to homogenize the mixture.

iv. Transfer the mixture to the corresponding cuvette and record the absorbance values A_1 at 253 nm for both tubes 30 seconds after timing.

v. Place the cuvettes into thermostatted compartments to maintain the temperature at 37°C. Were the thermostatted compartments unavailable, transfer the mixture in the cuvettes back to the test tube and warm the solution at 37°C for 20 min.

vi. Transfer the mixture back to the cuvettes if necessary and record the absorbance values A_2 20 minutes and 30 seconds after timing.

vii. Calculate the absorbance difference $\Delta A=A_2-A_1$.

II. Tissue Samples

a. Pre-treatment

i. Preparation of Pancreas Supernatant: Weigh the pancreas tissues precisely and add the reagent II with the ratio of 1g tissue to 9ml the reagent. Homogenate the mixture and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant for further measurement.

ii. Preparation of Small Intestine Mucosa and Chyme: Take freshly prepared or thawed small intestine and cut the intestine lengthwise along the intestine wall. Then collect the mucosa and chime clinging to the small intestine as the sample. Weigh the sample precisely and add reagent II with the ratio of 1g to 9ml. Homogenize the mixture and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant for further measurement and the supernatant can be preserved at -15°C for 15 days.

Note: The protein concentration of the supernatant should be measured simultaneously.

b. Procedures

Compositions (ml)	Blank	Sample
Reagent I Solution	1.5	1.5
	Pre-warm at 37°C for 5 min.	
Supernatant		a
Reagent II Solution	a	

Record the time after the addition of the supernatant. Mix the solution and for each type of solution, transfer it into a cuvette with 0.5cm path length. Record the absorbance value A_1 30 seconds after the addition of the supernatant. Place the cuvette into a thermostatted compartment at 37°C. Record the absorbance value A_2 at the timing of 20 minutes 30 seconds.

Note: It is recommended to do the pre-test before and under the circumstance of A_2-A_1 out of the range of 0.05-0.8, the sample should be diluted or concentrated (increase the sample amount) to receive results within the range recommended.

c. Detailed Procedures

i. Regulate both two cuvettes with double distilled water (DDW) with the spectrophotometer at 253 nm. (One cuvette for blank and the other one for sample measurement).

ii. Warm the reagent I solution at 37°C for more than 5 min.

iii. Add 1.5ml pre-warmed reagent I into both tubes and then weigh a ml (a is an arbitrary number) sample serum or reagent II solution into the corresponding tube. Start timing and shake the tube quickly to homogenize the mixture.

iv. Transfer the mixture to the corresponding cuvette and record the absorbance values A1 at 253 nm for both tubes 30 seconds after timing.

v. Place the cuvettes into thermostatted compartments to maintain the temperature at 37°C. Were the thermostatted compartments unavailable, transfer the mixture in the cuvettes to back to the test tube and warm the solution at 37°C for 20 minutes.

vi. Transfer the mixture back to the cuvettes if necessary and record the absorbance values A_2 20 minutes and 30 seconds after timing.

vii. Calculate the absorbance difference $\Delta A=A_2-A_1$.

5. Calculation Formula and Example

I. Serum Samples

a. Definition: One activity unit is defined as every 0.003 change in absorbance per minute caused by trypsin in 1 ml serum at 37°C and pH=8.

b. Formula

$$\frac{Activity}{U/ml} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{0.003} \div \frac{T}{20\ min} \times \frac{V_{Total}(1.5+a)}{V_{Sample}(a)} \div \frac{V_{Sample}}{a\ ml}$$

Note: $\Delta A=A_2-A_1$

c. Example

0.05ml serum taken from patients suffering pancreatitis was measured and the A_1 values were 0.721 and 1.011 respectively while the A_2 values were 0.721 and 1.088 respectively.

$$\frac{\text{Activity}}{\text{U/ml}} = \frac{\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}}{0.003} \div \frac{\text{T}}{20\ \text{min}} \times \frac{V_{\text{Total}}(1.5+\text{a})}{V_{\text{Sample}}(\text{a})} \div \frac{V_{\text{Sample}}}{\text{a ml}}$$

$$= \frac{(1.088-1.011)-(0.721-0.721)}{0.003} \div 20 \times \frac{1.55}{0.05} \div 0.05 = 7.957 \times 10^2 U/ml$$

II. Tissue Samples

a. Definition: One activity unit is defined as every 0.003 change in absorbance per minute caused by trypsin in 1 mg protein from tissues at 37°C and pH=8.

b. Formula

$$\frac{\text{Activity}}{\text{U/mg}} = \frac{\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}}{0.003} \div \frac{\text{T}}{20\ \text{min}} \times \frac{V_{\text{Total}}(1.5+\text{a})}{V_{\text{Sample}}(\text{a})} \div \frac{V_{\text{Sample}}}{\text{a ml}} \div \frac{C_{\text{Protein}}}{\text{mg/ml}}$$

c. Example

i. 10% mouse intestinal mucosa homogenate was prepared and 15 μl homogenate was taken and measured. The A_1 values and A_2 values were 0.721, 0.721, 1.009 and 1.224 for blank and sample tube respectively. Also, the protein concentration for the homogenate is 9.000 mg/ml.

$$\frac{Activity}{U/mg} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{0.003} \div \frac{T}{20\ min} \times \frac{V_{Total}(1.5+a)}{V_{Sample}(a)} \div \frac{V_{Sample}}{a\ ml} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{(1.224-1.009)-(0.721-0.721)}{0.003} \div 20 \times \frac{1.515}{0.015} \div 0.015 \div 9.000$$

$$= 2.681 \times 10^3\ \text{u/mg}$$

ii. 10% crucian intestinal mucosa homogenate was prepared and 15 μl homogenate was taken and measured. The A_1 values and A_2 values were 0.721, 0.721, 0.921 and 1.051 for blank and sample tube respectively. Also, the protein concentration for the homogenate is 8.180 mg/ml.

$$\frac{Activity}{U/mg} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{0.003} \div \frac{T}{20\,min} \times \frac{V_{Total}(1.5+a)}{V_{Sample}(a)} \div \frac{V_{Sample}}{a\,ml} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{(1.051 - 0.921) - (0.721 - 0.721)}{0.003} \div 20 \times \frac{1.515}{0.015} \div 0.015 \div 8.180$$

$$= 1.783 \times 10^3\,\mathrm{u/mg}$$

iii. 10% mouse pancreas homogenate prepared and 50 μl homogenate was taken and measured. The A_1 values and A_2 values were 0.721, 0.721, 1.103 and 1.166 for blank and sample tube respectively. Also, the protein concentration for the homogenate is 7.340 mg/ml.

$$\frac{Activity}{U/mg} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{0.003} \div \frac{T}{20\,min} \times \frac{V_{Total}(1.5+a)}{V_{Sample}(a)} \div \frac{V_{Sample}}{a\,ml} \div \frac{C_{Protein}}{mg/ml}$$

$$= \frac{(1.166 - 1.103) - (0.721 - 0.721)}{0.003} \div 20 \times \frac{1.55}{0.05} \div 0.05 \div 7.340$$

$$= 8.869 \times 10^1\,\mathrm{u/mg}$$

6. Sensitivity

The lowest trypsin activity that can be detected by this kit is 3U/mg (for tissues) or 30U/ml (for serum).

Appendix I: Reaction Curve Establishment

1. Sample Source

Mouse intestinal mucosa was taken and 10% homogenate was prepared. The homogenate was then centrifuged at 3,500 rpm for 10 min and supernatant was extracted for measurement.

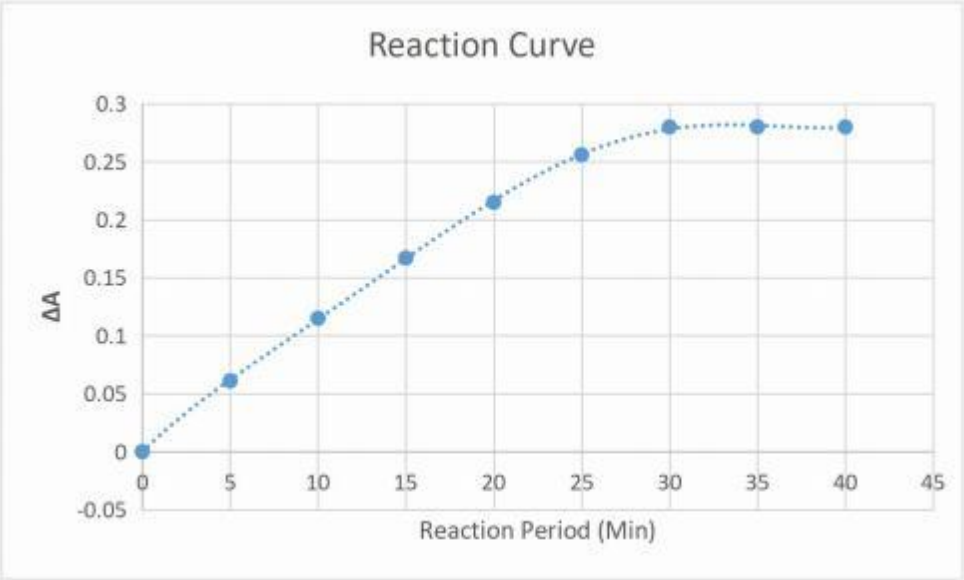
2. Procedures

Compositions (ml)	Blank	Sample
Reagent I Solution	1.5	1.5
Pre-warm at 37°C for 5 min.		
Supernatant		0.015
Reagent II Solution	0.015	

Record the time after the addition of the supernatant. Mix the solution and for each type of solution, transfer it into a cuvette with 0.5cm path length. Record the absorbance value A_1 30 seconds after the addition of the supernatant. Place the cuvette in a thermostatted compartment at 37°C. Record the absorbance value A_2 at the timing of 20 minutes 30 seconds.

3. Results

Timing	Reaction Period (Min)	A_2	ΔA
30″	0	1.009	0
5′ 30″	5	1.070	0.061
10′ 30″	10	1.124	0.115
15′ 30″	15	1.176	0.167
20′ 30″	20	1.224	0.215
25′ 30″	25	1.265	0.256
30′ 30″	30	1.289	0.280
35′ 30″	35	1.289	0.280
40′ 30″	40	1.289	0.280


Reaction Curve
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
ΔA
0
5
10
15
20
25
30
35
40
45
Reaction Period (Min)