(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Trypsin Activity Assay Kit

Catalog No.: BC00152

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Trypsin Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, pancreas, small intestine
Assay Type	Quantitative
Detection Instrument	Microplate reader (optimal detection wavelength 253 nm)

Product Introduction

In most patients with acute pancreatitis and chronic renal failure, trypsin levels are significantly elevated, as are in more than half of patients with pancreatic cancer and chronic pancreatitis. However, trypsin levels are also elevated in 20% of patients with non-pancreatic abdominal pain, particularly those with cholecystitis and perforated duodenal ulcers. In chronic pancreatitis, biliary tract diseases, and chronic cholecystitis, trypsin levels are decreased.

Principle

Trypsin can catalyze the hydrolysis of the ester chain of the substrate arginine ethyl ester, causing its absorbance value to increase at 253 nm. The enzyme activity can be calculated based on the change in absorbance.

Components

Components	Name	Size	Storage
Reagent 1	Substrate powder	Powder × 2 tubes	2~8°C
	Substrate dilution	30 mL × 3 bottles	2~8°C

	Preparation of substrate application solution: Add one vial of powder to one bottle of diluent immediately before use, and dissolve thoroughly. The substrate application solution must be prepared fresh for use and is effective for 24 hours at 2–8°C.		
Reagent 2	Homogenizing medium	60 mL × 2 bottles	2~8℃

Storage

The kit has a shelf life of 6 months

Preparation before the experiment

Sample processing

1. Serum (plasma): measured directly.
2. Preparation of pancreatic supernatant: Accurately weigh pancreatic tissue, add 9 times the volume of homogenizing medium at a weight (g): volume (mL) ratio of 1:9, mechanically homogenize under ice bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for analysis.
3. Preparation of small intestinal elk and mucosal supernatant: Take fresh or completely thawed small intestine, cut it longitudinally along the intestinal wall, and scrape off the elk and mucosa with a small blade (the entire operation is performed on a plastic wrap in a refrigerator). Accurately weigh the elk and mucosa, and add the sample homogenizing medium to the elk and mucosa at a weight-to-volume ratio of 1:9 (tissue:sample homogenizing medium). Mechanically homogenize under ice bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for analysis. Store the supernatant frozen at -20℃ or below; it is valid for 15 days.

Note: The protein content of the homogenate supernatant must be determined simultaneously (protein content determination kit BC00006).

Operation process

1. Set the UV spectrophotometer to 253 nm, use a 0.5 cm optical path quartz cuvette, and zero it with double-distilled water; (prepare two quartz cuvettes, one for zeroing and one for measurement);
2. Preheat the trypsin substrate solution to 37°C for at least 5 minutes.
3. Add a mL of fresh serum (plasma) to the corresponding numbered test tube, and add 1.5 mL of trypsin substrate working solution to the test tube. Mix quickly and start timing. (For the blank tube, take two a mL of reagent, add 1.5 mL of working solution to the test tube, mix quickly and start timing. Other operations are the same as for the test tube.)
4. Quickly pour into a quartz cuvette, use a UV spectrophotometer to measure the color at 253 nm, and read the absorbance value A1 after 30 seconds;
5. Pour this colorimetric solution into the original test tube and place it in a water bath at 37°C for 20 minutes. Then quickly pour it into a quartz cuvette and read the absorbance value A2 at 20 minutes and 30 seconds.
6. Calculate the difference in absorbance between the two measurements (ΔA = A2 - A1).

Serum (plasma) sample handling procedure:

Reagents(mL)	Blank tube	Test tube
Trypsin substrate application solution	1.5	1.5
Preheat to 37 ℃ for 5 minutes		
Sample	--	a
Reagent 2	a	--

Start timing while mixing the sample and substrate solution. After mixing, pour the mixture into a quartz cuvette with a 0.5 cm optical path and measure the absorbance OD value at 253 nm. Record the absorbance OD value A1 at 30 seconds. Place the cuvette in the cuvette without moving it, and keep the temperature of the cuvette constant at 37°C. If the temperature of the cuvette cannot be kept constant at 37°C, pour the reaction solution from the cuvette into a pre-numbered test tube

and place it in a 37°C water bath for 20 minutes. Record the absorbance OD value A2 at 20 minutes and 30 seconds and calculate ΔA = A2 - A1.

Organizing sample handling procedures:

Reagents(mL)	Blank tube	Test tube
Trypsin substrate application solution	1.5	1.5
Preheat to 37 ℃ for 5 minutes		
Sample	--	0.015
Reagent 2	0.015	--

Start timing while mixing the sample and substrate solution. After mixing, pour the mixture into a quartz cuvette with a 0.5 cm optical path and measure the absorbance OD value at 253 nm. Record the absorbance OD value A1 at 30 seconds. Place the cuvette in the cuvette without moving it, and keep the temperature of the cuvette constant at 37°C. If the temperature of the cuvette cannot be kept constant at 37°C, pour the reaction solution from the cuvette into a pre-numbered test tube and place it in a 37°C water bath for 20 minutes. Record the absorbance OD value A2 at 20 minutes and 30 seconds and calculate ΔA = A2 - A1.

Note:

1. If ΔA is less than or equal to 0.050 during the preliminary test, the sample size or concentration should be increased accordingly before the test is repeated.
2. If the measured ΔA is greater than 0.250 during the preliminary test, the sample should be diluted with sample homogenization medium (reagent 2) and measured again.

Result calculation

1、Serum (plasma) trypsin activity calculation :

Unit definition: Under conditions of pH 8.0 and 37°C, one unit of enzyme activity is defined as the change in absorbance of trypsin in each milliliter of serum (plasma) by 0.003 per minute.

$$\text{Trypsin Activity (U/mL)} = \frac{\triangle A_{test} - \triangle A_{blank}}{0.003} \times \frac{V_{reaction\ total}}{V_{sample}} \times \frac{1}{V_{sample}} \div T$$

2、Calculation of trypsin activity in tissue homogenate :

Unit definition: Under conditions of pH 8.0 and 37°C, one unit of enzyme activity is defined as the change in absorbance of trypsin in each milligram of protein by 0.003 per minute.

$$\underset{(\mathrm{U/mgprot})}{\text{Trypsin Activity}} = \frac{\triangle A_{test} - \triangle A_{blank}}{0.003} \times \frac{V_{reaction\ total}}{V_{sample}} \div T \div (Cpr \times V_{sample})$$

Annotation

Under normal conditions, the absorbance of the blank tube remains stable and unchanged.

$V_{reaction\ total}$: Total volume of the reaction system, 1.5 + a (mL);

V_{sample}: Sample volume, a (mL);

T: reaction time, 20 min.

Cpr : Protein concentration in homogenate sample, mgprot/mL (prot refers to protein) .

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.