Trypsin Activity Assay Kit

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

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: BC2315

Size:100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I A	Liquid 50 μL×1	-20℃
Reagent I B	Liquid 2 mL×1	2-8℃
Reagent II	Liquid 0.22 mL×1	2-8℃
Reagent III	Liquid 30 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Preparation of Reagent I working liquid: Before use, the Reagent I working liquid was prepared according to the sample size in the ratio of Reagent I A: Reagent I B = 10 μL: 210 μL(220 μL, 22T).For long-term storage, please keep at -20°C and avoid repeated freezing and thawing.

2. Reagent II: Reagent is placed in EP tube inside reagent vial.As Reagent II solidifies when exposed to cold, please place it at room temperature for a period of time to allow it to fully dissolve before use (it can also be pre-packaged for storage). Prior to use, centrifuge the liquid to the bottom of the tube (a handheld centrifuge can be used).

3. Preparation of Reagent II working liquid: Before the experiment, part of the Reagent III was separately placed in 37℃ water bath and preheated for more than 10min. Before use, the Reagent II working liquid was prepared in the ratio of Reagent II:Reagent III = 10μL: 990μL(1 mL, 5T). **Prepare the working solution of Reagent II just before adding the samples based on the volume of the samples, and do not store it for later use.**

4. Standard: 5 μmol/mL p-Nitroaniline standard

5. Preparation of 0.08 μmol/mL Standard : Take 50 μL of the 5 μmol/mL standard solution and add it to 200 μL of Reagent III to prepare a 1 μmol/mL standard solution. Then, take 80 μL of the 1 μmol/mL standard solution and add it to 920 μL of Reagent III to prepare a 0.08 μmol/mL standard solution, ready for use.

Product Description:

Trypsin (EC 3.4.4.4) selectively hydrolyzes peptide bonds in denatured proteins that are

formed by the carboxyl groups of lysine or arginine, making it an important digestive enzyme. Additionally, trypsin is

widely used in the adjunctive treatment of local edema, hematoma, and abscesses resulting from conditions such as empyema, hemothorax, surgical inflammation, ulcers, and traumatic injuries.

Trypsin catalyzes the substrate BAPNA to generate p-nitroaniline (p-NA), which has a characteristic absorption peak at a wavelength of 405 nm. Since the absorbance of p-NA is directly proportional to its concentration, the enzyme activity of trypsin can be calculated by measuring the amount of p-NA produced per unit of time.

Reagents and Equipment Required but Not Provided:

Mortar/homogenizer, ice, desk centrifuge, visible spectrophotometer/microplate reader, micro quartz cuvette/96 well plate, adjustable pipette and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue: Add 1 mL of Extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 10000 rpm

for 10 minutes at 4℃, take the supernatant and put it on ice for test.

II. Determination procedure:

1. Preheat visible spectrophotometer/microplate for 30 minutes, adjust the wavelength to 405 nm, set the counter to zero with distilled water.
2. Preheat Reagent III in water-bath at 37℃ for 10 minutes. Prepare the working solution of Reagent II immediately **before adding the samples (prepare only what is needed for immediate use, do not store**).
3. Add the following reagents to micro quartz cuvette/96 well flat-bottom UV plate:

Reagent name (μL)	Test tube (A_T)	Control tube (A_C)	Standard tube	Blank tube (A_B)
Sample	20	-	-	-
Extract solution	-	20	-	-
Reagent I working	10	10	-	-
Reagent II working	170	170	-	-
Standard			200	-
Reagent III			-	200

Add the working solution of Reagent II and start timing simultaneously. After mixing thoroughly and quickly, measure the absorbance at 405nm at 30 seconds and record it as A1. Let the reaction proceed at 37°C for 1 hour, then quickly measure the absorbance at 1 hour and 30 seconds and record it as A2. ΔA_T= ($A2_T$-$A1_T$)-($A2_C$-$A1_C$). The control tubes only need to be measured 1-2 times. If using a micro glass cuvette for measurement, the reagents mentioned above can be directly added into the cuvette. After	Directly measure the absorbance at 405nm, recording them as A_S and A_B. ΔA_S= A_S-A_B. The blank and standard tubes only need to be measured 1-2 times.

III. Calculation:

(1) Calculate by sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of p-Nitroaniline per hour every milligram of protein.

Trypsin Activity (U/mg Prot) =$\Delta A_T \div \Delta A_S \div \times C_S \times Vrv \div (Cpr \times Vs) \div T \times F$

$=0.8 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

(2) Calculate by sample mass:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of p-Nitroaniline per hour every gram of sample.

Trypsin Activity (U/g) =$\Delta A_T \div \Delta A_S \div \times C_S \times Vrv \div (W \times Vs \div Vsv) \div T \times F$

$=0.8 \times \Delta A_T \div \Delta A_S \div W \times F$

C_S: Standard control p-Nitroaniline concentration, 0.08 μmol/mL;

Vrv: Total reaction volume,0.2 mL

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

Vs: The volume of the sample added to the reaction system, 0.02 mL;

T: Reaction time (min), 1 hour;

Vsv: Extract solution volume, 1 mL;

W: Sample weight(g);

F: Sample dilution factor.

Note:

1. Before the experiment, conduct a preliminary test with 1 to 2 samples to ensure that the ΔA_T is between 0.01 and 1.5; if the ΔA_T >1.5, it is recommended to dilute the sample with the extraction solution before testing. If the ΔA_T <0.01, the sample volume in the operation table can be increased (while reducing the volume of Reagent II working solution). Remember to adjust the calculation formula accordingly.

Experimental example:

1. Take 0.1037g of rat pancreas and add 1 mL of Extract solution for ice bath homogenization. After centrifugation, the supernatant is diluted by a factor of 2 with the extraction solution, and then the measurement steps are followed. The enzyme activity is calculated as follows:

$\Delta A_T=(A2_T-A1_T)-(A2_C-A1_C)=(0.988-0.320)-(0.240-0.158)=0.586$, $A_S=A_S-A_B=0.515-0.047=0.468$.

Calculated based on the sample mass:Trypsin Activity (U/g) ==$0.8\times\Delta A_T\div\Delta A_S\div W\times F=19.319$ U/g

Recent Product Citations：

[1] V. Kuzmina, N. V. Ovchinnikova, and S. M. Tolpygo. Serum Activity of Proteolytic Enzyme Trypsin in Rats under Conditions of Water and Food Deprivation[J]. Bulletin of Experimental Biology and Medicine, 2023, 175(5): 540-544.

Related Products：

BC2280/BC2285 Acid Protease (ACP) Activity Assay Kit

BC2290/BC2295 Neutral Protease (NP) Activity Assay Kit

BC2320/BC2325 Pepsin Activity Assay Kit

BC2330/BC2335 Chymotrypsin Activity Assay Kit