Acidic Proteinase (ACP)Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC2280

Size: 50T/24S

Components:

Extract solution: Liquid 35 mL×1, store at 2-8℃.

Reagent I: Liquid 10 mL×1, store at 2-8℃.

Reagent II: Powder×1, store at 2-8℃; add 10 mL of Reagent V before use. Put it in boiling water bath and dissolve it by magnetic stirring.

Reagent III: Liquid 50 mL×1, store at 2-8℃;

Reagent IV: Liquid 10 mL×1, store at 2-8℃;

Reagent V: Liquid 15 mL×1, store at 2-8℃;

Standard: Liquid 1 mL×1, 20 μmol/mL tyrosine standard solution, store at 2-8℃;

Product Description:

ACP is an enzyme that catalyzes the hydrolysis of proteins in acidic environments. The enzyme is mainly used in alcohol fermentation, beer brewing, fur softening, fruit wine clarification, soy sauce brewing, feed and so on.

In acidic condition, ACP can catalyzes the hydrolysis of casein to produce tyrosine. In alkaline condition, tyrosine reduces phosphomolybdic acid compound to tungsten blue which has a characteristic absorption peak at 680 nm, and the activity of ACP is calculated by measuring its absorbance increase.

Required but not provided:

Mortar/homogenizer, desk centrifuge, spectrophotometer, water bath, magnetic stirrer, transferpettor, 1.5 mL centrifuge tube, 1 mL glass cuvette and distilled water.

Procedure:

I. Sample preparation

Add 1 mL Extract solution to 0.1 g tissue, fully grind on ice. Centrifuge at 4℃ 10000 rpm for 10 minutes. Take the supernatant as crude enzyme. Place the supernatant on ice for test. It also can add 1 mL Extract solution to 0.1 g enzyme preparation. Put it on ice to be tested.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 680 nm, set the counter to zero with distilled water.
2. Incubate Reagent I, II, III at 30℃ water bath for 30 minutes.
3. Preparation of standard solution: before use, dilute 20 μmol/mL standard solution with distilled water 80 times to 0.25 μmol/mL standard solution for use now.
4. Sample determination (add the following reagents in 1.5 mL EP tube in turn).

Reagent Name (μL)	Contrast tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Crude enzyme	100	100		
Extract solution	100	100		
Reagent I	200			
Reagent II		100		
Mix thoroughly, incubate at 30℃ water bath for 10 minutes.				
Reagent I		200		
Reagent II	100			
Mix thoroughly. Centrifuge at 4℃ 10000 rpm for 10 minutes. Take the supernatant.				
Supernatant	200	200		
Distilled water			200	
Standard				200
Reagent III	1000	1000	1000	1000
Reagent IV	200	200	200	200
Mix thoroughly, incubate at 30℃ water bath for 20 minutes.				

Add 1 mL the reaction solution to 1 mL glass cuvette, detect the absorbance at 680 nm, record as A_C, A_T, A_B, A_S.

III.Calculation

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per minute at 30℃ every mg protein.

ACP (U/mg prot)=$C_S\times(A_T-A_C)\div(A_S-A_B)\times V1\div(Cpr\times V2)\div T=0.125\times(A_T-A_C)\div(A_S-A_B)\div Cpr$

2. Sample fresh weight.

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of tyrosine in the reaction system per minute at 30℃ every g sample.

ACP (U/g weight)=$C_S\times(A_T-A_C)\div(A_S-A_B)\times V1\div(W\times V2\div V3)\div T=0.125\times(A_T-A_C)\div(A_S-A_B)\div W$

C_S: Standard solution, 0.25 μmol/mL;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g;

V1: Reaction total volume, 0.5 mL;

V2: Crude enzyme solution volume, 0.1 mL;

V3: Total volume of crude enzyme, 1 mL;

T: Reaction time, 10 minutes.

Note:

If reaction is weak and (A_T-A_C) is small, prolong the water bath time of the first step (20-30

minutes), and the formula should be modified when calculating the enzyme activity.

Experimental example:

1. Take 0.1g mouse liver, add 1 mL of Extract solution, grind it on ice, centrifuge at 4℃ for 10min at 10000rpm, take supernatant and put it on ice, then operate according to the determination steps, use 96 well plate to measure and calculate: A_T = 0.537, A_C = 0.481, A_S= 0.39, A_B= 0.005
ACP activity (U/g mass) = 0.125 × (A_T-A_C) ÷ (A_S-A_B) ÷ W = 0.182 U/g mass.

Recent Product Citations:

[1] Xin-Bin, Gu, Xin, et al. Hematopoietic Substrate-1-Associated Protein X-1 Regulates the Proliferation and Apoptosis of Endothelial Progenitor Cells Through Akt Pathway Modulation[J]. Stem Cells, 2017. (IF 5.614)

[2] Shijun Wang, Yunfei Cao, Zuqing Yang, et al. MicroRNA-93-5p increases multidrug resistance in human colorectal carcinoma cells by downregulating cyclin dependent kinase inhibitor 1A gene expression. Oncology Letters. December 2016. (IF 1.874)

Related Products:

BC2290/BC2295 Neutral Proteinase(NP) Activity Assay Kit
BC2300/BC2305 Alkali Proteinase(AKP) Activity Assay Kit