Acidic Proteinase (ACP)Activity Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: NA0466

Size: 100T/48S

Components:

Extract solution: Liquid 55 mL×1, store at 4°C.

Reagent I: Liquid 4 mL×1, store at 4°C;

Reagent II: Powder×1, store at 4°C and protect from light; add 4 mL of Reagent V before use. Put it in boiling water bath and dissolve it by magnetic stirring.

Reagent III: Liquid 20 mL×1, store at 4°C;

Reagent IV: Liquid 4 mL×1, store at 4°C;

Reagent V: Liquid 5 mL×1, store at 4℃;

Standard: Liquid 1 mL×1, 20 μmol/mL tyrosine standard solution, store at 4°C;

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 catalyze the hydrolysis of casein to produce tyrosine; in alkaline condition, tyrosine reduces phosphomolybdic acid compound to tungsten blue which has 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/microplate reader, water bath, micro glass cuvette/96 well flat-bottom plate, magnetic stirrer, transferpettor, 1.5 mL centrifuge tube and distilled water.

Procedure:

I. Sample preparation

Add 1 mL Extract solution to 0.1 g tissue, fully grind on ice. Centrifuge at 4°C and 10000rpm 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/microplate reader for 30 minutes, adjust wavelength to 680 nm, set the counter to zero with distilled water.
2. Incubate Reagent I, II, III at 30°C 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	20	20		
Reagent I	40			
Reagent II		40		
Mix thoroughly, incubate at 30℃ water bath for 10 minutes.				
Reagent I		40		
Reagent II	40			
Mix thoroughly. Centrifuge at 4℃ and 10000 rpm for 10 minutes. Take the supernatant.				
Supernatant	40	40		
Distilled water			40	
Standard				40
Reagent III	200	200	200	200
Reagent IV	40	40	40	40
Mix thoroughly, incubate at 30℃ water bath for 20 minutes.				

Add 200 μL crude enzyme to micro glass cuvette/96 well flat-bottom plate, 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, 0.1 mL;

V2: Crude enzyme solution enzyme, 2 μL=2×10^{-2} 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 = 0.303, A_C = 0.271, A_S= 0.253, A_B= 0.044

ACP activity (U/g mass) = 0.125 × (A_T-A_C) ÷ (A_S-A_B) ÷ W = 0.191 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：

NA0706/NA0465 Neutral Proteinase(NP) Activity Assay Kit

NA0705/NA0464 Alkali Proteinase(AKP) Activity Assay Kit