Alkaline Protease (AKP) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC2300

Size: 50T/24S

Components:

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

Reagent I: Powder×1, store at 2-8℃; add 10 mL distilled water before use.

Reagent II: Powder×1, store at 2-8℃ and protect from light; add 10 mL Extract solution 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℃;

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

Product Description:

AKP is a serine protease, which catalyzes the hydrolysis of protein peptide bond in alkaline condition. In addition, the enzyme can hydrolyze ester bond and amide bond. It has the function of trans ester and trans peptide. The enzyme is one of the main industrial enzymes, which is widely used in pharmaceutical, silk, food, leather and other industries.

In alkaline condition, AKP hydrolyzes casein to produce tyrosine. In alkaline condition, tyrosine reduced phosphomolybdic acid to tungsten blue. Tungsten blue has a characteristic absorption peak at 680 nm. The activity of AKP can be calculated by measuring the increasing rate of 680 nm absorbance.

Required but not provided:

Mortar/homogenizer, desk centrifuge, spectrophotometer, water bath, magnetic stirrer, transferpettor, 1.5 mL EP 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 40℃ water bath for 30 minutes.
3. Sample determination (add the following reagents in 1.5 mL EP tube in turn).

Reagant Name (μL)	Contrast tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Crude enzyme	100	100		

Reagent I	200			
Reagent II		200		
Mix thoroughly, incubate at 40℃ water bath for 10 minutes.				
Reagent I		200		
Reagent II	200			
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 40℃ 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 40℃ every mg protein.

AKP (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 40℃ every g sample.

AKP (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, D-value of 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.