Alkaline Protease (AKP) Activity Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: NA0464

Size: 100T/48S

Components:

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

Reagent I: Powder×1, store at 4℃; add 4 mL of distilled water before use.

Reagent II: Powder×1, store at 4℃; add 3 mL of Extract solution before use. Put it in boiling water bath and dissolve it by magnetic stirring.

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

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

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

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/microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, 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℃ 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/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 40℃ 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.

4. 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	20	20		
Reagent I	40			
Reagent II		40		
Mix thoroughly, incubate at 40℃ water bath for 10 minutes.				
Reagent I		40		
Reagent II	40			
Mix thoroughly. Centrifuge at 4℃ 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 40℃ water bath for 20 minutes.				

Add 200 μL reaction solution 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. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$. Blank tube only need to be test one or two times.

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.

$AKP(U/mg\ prot)=C_S\times\Delta A_T\div\Delta A_S\times V1\div(Cpr\times V2)\div T=0.125\times\Delta A_T\div\Delta A_S\div Cpr$

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.

$AKP(U/g\ weight)=C_S\times\Delta A_T\div\Delta A_S\times V1\div(W\times V2\div V3)\div T=0.125\times\Delta A_T\div\Delta A_S\div W$

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 volume, 20 μL=2×10^{-2} 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.

Related Products：

NA0707/NA0466 Acidic Proteinase(ACP) Activity Assay Kit

NA0706/NA0465 Neutral Proteinase(NP) Activity Assay Kit