Neutral Proteinase(NP) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0706

Size: 50T/24S

Components:

Extract solution: Liquid 35 mL×1, store at 4℃.

Reagent I: Powder×1, store at 4℃; add 10 mL of distilled water before use.

Reagent II: Powder×1, store at 4℃; add 10 mL of Extract solution before use. Put it in boiling water bath and dissolve it by magnetic stirring.

Reagent III: Liquid 50 mL×1, store at 4℃;

Reagent IV: Liquid 10 mL×1, store at 4℃;

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

Product Description:

NP catalyze the hydrolysis of protein under certain temperature and neutral PH conditions. It has the characteristics of safety, non-toxicity, strong hydrolysis ability and wide range of action. So NP is often used in the production of food, feed, cosmetics and nutritional health products.

In neutral conditions, NP can catalyze the hydrolysis of casein to produce tyrosine. In alkaline condition, tyrosine reduces phosphomolybdic acid compounds to tungsten blue, which has a characteristic absorption peak at 680 nm.

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 of Extract solution to 0.1g of tissue, fully grind on ice. Centrifuge at 4℃ 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 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

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		
Reagent I	200			
Reagent II		200		
Mix thoroughly, incubate at 30℃ 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 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.

NP (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.

NP (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 rabbit spleen, add 1ml extract, grind it on ice, centrifuge at 4℃ for 10 min at 10000rpm, take the supernatant, that is, crude enzyme solution, and put it on ice. Then operate according to the determination steps, and calculate $\Delta A_T=A_T-A_C$ = 0.195-0.175 = 0.02, $\Delta A_S =A_S-A_B$ = 0.408-0 = 0.408. The enzyme activity is calculated according to the sample mass

NP activity (U/g mass) = $0.125\times\Delta A_T \div \Delta A_S \div W$ = 0.125×0.02 ÷ 0.408 ÷ 0.1 = 0.0613 U/g mass.

Related Products：

NA0707/NA0466 Acidic Proteinase(ACP) Activity Assay Kit

NA0705/NA0464 Alkali Proteinase(AKP) Activity Assay Kit