Neutral Proteinase (NP) Activity Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: BC2295

Size: 100T/48S

Components:

Extract solution: Liquid 55 mL×1, store at 2-8℃.

Reagent I: Powder×1, store at 2-8℃; add 4 mL of distilled water before use.

Reagent II: Powder×1, store at 2-8℃; add 4 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 2-8℃;

Reagent IV: Liquid 4 mL×1, store at 2-8℃;

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

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/microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, magnetic stirrer, transferpettor, 1.5 mL centrifuge tube, ice 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 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.

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4. Sample determination (add the following reagents in 1.5 mL EP tube in turn).

<table>
<tr><th>Reagent Name (μL)</th><th>Contrast tube (A_C)</th><th>Test tube (A_T)</th><th>Blank tube (A_B)</th><th>Standard tube (A_S)</th></tr>
<tr><td>Crude enzyme</td><td>20</td><td>20</td><td rowspan="8">-</td><td rowspan="8">-</td></tr>
<tr><td>Reagent I</td><td>40</td><td>-</td></tr>
<tr><td>Reagent II</td><td>-</td><td>40</td></tr>
<tr><td colspan="3">Mix thoroughly, incubate at 30℃ water bath for 10 minutes.</td></tr>
<tr><td>Reagent I</td><td>-</td><td>40</td></tr>
<tr><td>Reagent II</td><td>40</td><td>-</td></tr>
<tr><td colspan="3">Mix thoroughly. Centrifuge at 4℃ 10000 rpm for 10 minutes. Take the supernatant.</td></tr>
<tr><td>Supernatant</td><td>40</td><td>40</td></tr>
<tr><td>Distilled water</td><td>-</td><td>-</td><td>40</td><td>-</td></tr>
<tr><td>Standard</td><td>-</td><td>-</td><td>-</td><td>40</td></tr>
<tr><td>Reagent III</td><td>200</td><td>200</td><td>200</td><td>200</td></tr>
<tr><td>Reagent IV</td><td>40</td><td>40</td><td>40</td><td>40</td></tr>
<tr><td colspan="5">Mix thoroughly, incubate at 30℃ water bath for 20 minutes.</td></tr>
</table>

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. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$.

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) $=C_S\times\Delta A_T\div\Delta A_S\times V1\div(Cpr\times V2)\div T=0.125\times\Delta A_T\div\Delta A_S\div Cpr$

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 30℃ every g sample.

NP (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.

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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 1 mL of Extract solution, grind it on ice, centrifuge at 4℃ for 10min at 10000rpm, take the supernatant, namely crude enzyme solution, and put it on ice, then operate according to the determination steps, calculate $\Delta A_T = A_T - A_C = 0.132 - 0.123 = 0.009$, $\Delta A_S = A_S - A_B = 0.259 - 0.045 = 0.214$ with 96 well plate. The enzyme activity is calculated according to the sample mass

NP activity (U/g mass) = $0.125 \times \Delta A_T \div \Delta A_S \div W = 0.125 \times 0.009 \div 0.214 \div 0.1 = 0.0526$ U/g mass.

Related Products:

BC2280/BC2285 Acidic Proteinase (ACP) Activity Assay Kit
BC2300/BC2305 Alkali Proteinase (AKP) Activity Assay Kit

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