# Neutral Protease(NP) Activity Assay Kit

Product Code: 112037

**Neutral Protease (NP) Activity Assay Kit - Micro Method**

## Product Information

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| Product Code | 112037 |
| Product Name | Neutral Protease Activity Assay Kit - Micro Method |
| Size | 100T |

## Product Introduction

Neutral protease catalyzes the hydrolysis of proteins under suitable temperature and neutral pH conditions. It is commonly used in food, feed, cosmetics, and nutritional health product applications because it is safe, non-toxic, has strong hydrolysis ability, and has a wide range of action.

Under neutral conditions, neutral protease catalyzes the hydrolysis of casein to produce tyrosine. Under alkaline conditions, tyrosine reduces the phosphomolybdic acid compound to form tungsten blue, which has a characteristic absorption peak at 680 nm.

Actual readings may vary depending on test conditions and instruments.

## Package Contents

| Code | Component | Quantity |
|---|---|---|
| 112037.1 | Reagent One | 1 bottle |
| 112037.2 | Reagent Two | 1 bottle |
| 112037.3 | Reagent Three | 1 bottle |
| 112037.4 | Reagent Four | 1 bottle |
| 112037.5 | Reagent Five | 1 bottle |
| 112037.6 | Standard | 1 bottle |
| 112037.m | Instruction Manual | 1 copy |

## Quality and Safety Information

| Component | Quality Standard | Main Toxicity |
|---|---|---|
| Reagent One | -- | -- |
| Reagent Two | -- | -- |
| Reagent Three | -- | -- |
| Reagent Four | -- | -- |
| Reagent Five | -- | -- |
| Standard | -- | -- |

## Transportation and Storage

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|---|---|
| Transportation | Shipped with ice packs. |
| Storage | Store at 2-8°C, protected from light. |
| Shelf Life | 180 days. |

## Instructions for Use

## 1. Preparation of Crude Enzyme Extract

### 1.1 Tissue Samples

Use tissue mass (g) to Reagent I volume (mL) at a ratio of 1:5-10. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Reagent I.

1. Homogenize in an ice bath.
2. Centrifuge at 8000g, 4°C for 10 min.
3. Collect the supernatant as the crude enzyme extract.

### 1.2 Bacteria and Fungi

Use cell number (10^4 cells) to Reagent I volume (mL) at a ratio of 500-1000:1. It is recommended to add 500 × 10^4 cells to 1 mL Reagent I.

1. Disrupt the cells by sonication in an ice bath: power 300 W, sonicate for 3 seconds, interval 7 seconds, total time 3 min.
2. Centrifuge at 8000g, 4°C for 10 min.
3. Collect the supernatant and place it on ice for testing.

### 1.3 Serum or Culture Medium

Measure directly.

## 2. Reagent Preparation

Before use, add 10 mL Reagent I to Reagent III and dissolve with magnetic stirring in a boiling water bath.

> To prevent water evaporation, the beaker may be covered with a layer of plastic wrap. Heating generally takes 15-30 minutes. This reagent is supersaturated; insoluble particles may remain after thorough mixing and do not affect use.

## 3. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for 30 min. Set the wavelength to 680 nm and zero with distilled water.
2. Place Reagent II, Reagent III, and Reagent IV in a 30°C water bath and incubate for 30 min.

### 3.1 Control Tube

1. In a 0.5 mL EP tube, add 20 μL crude enzyme solution and 40 μL Reagent II.
2. Mix well and incubate in a 30°C water bath for 10 min.
3. Add 40 μL Reagent III and mix well.
4. Centrifuge at 8000g, 4°C for 10 min.
5. Transfer 40 μL supernatant to a new EP tube.
6. Add 200 μL Reagent IV and 40 μL Reagent V.
7. Mix well and incubate in a 30°C water bath for 20 min.
8. Transfer 200 μL to a micro glass cuvette or 96-well plate.
9. Measure absorbance at 680 nm and record as $A_{control}$.

### 3.2 Assay Tube

1. In a 0.5 mL EP tube, add 20 μL crude enzyme solution and 40 μL Reagent III.
2. Mix well and incubate in a 30°C water bath for 10 min.
3. Add 40 μL Reagent II and mix well.
4. Centrifuge at 8000g, 4°C for 10 min.
5. Transfer 40 μL supernatant to a new tube.
6. Add 200 μL Reagent IV and 40 μL Reagent V.
7. Mix well and incubate in a 30°C water bath for 20 min.
8. Transfer 200 μL to a micro glass cuvette or 96-well plate.
9. Measure absorbance at 680 nm and record as $A_{assay}$.

Unlike the control tube, add Reagent III first, then add Reagent II.

### 3.3 Blank Tube

1. In a tube, add 40 μL distilled water, 200 μL Reagent IV, and 40 μL Reagent V.
2. Mix well and incubate in a 30°C water bath for 20 min.
3. Transfer 200 μL to a micro glass cuvette or 96-well plate.
4. Measure absorbance at 680 nm and record as $A_{blank}$.

### 3.4 Standard Tube

1. In a tube, add 40 μL standard, 200 μL Reagent IV, and 40 μL Reagent V.
2. Mix well and incubate in a 30°C water bath for 20 min.
3. Transfer 200 μL to a micro glass cuvette or 96-well plate.
4. Measure absorbance at 680 nm and record as $A_{standard}$.

The blank tube and standard tube need to be measured only once.

## Calculation of Neutral Protease Activity

### 1. Calculation Based on Sample Protein Concentration

Unit definition: At 30°C, the hydrolytic production of 1 nmol tyrosine per milligram of protein per minute is 1 enzyme activity unit.

NP activity (nmol/min/mg prot) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div (Cpr \times V1) \div T$

NP activity (nmol/min/mg prot) = $125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \div Cpr$

### 2. Calculation Based on Sample Mass

Unit definition: At 30°C, the catalytic hydrolytic production of 1 nmol tyrosine per gram of sample per minute is 1 enzyme activity unit.

NP activity (nmol/min/g fresh weight) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div (W \times V1 \div V2) \div T$

NP activity (nmol/min/g fresh weight) = $125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \div W$

### 3. Calculation by Liquid Volume

Unit definition: At 30°C, the catalytic hydrolysis of each milliliter of sample per minute produces 1 nmol tyrosine as 1 enzyme activity unit.

NP activity (nmol/min/mL) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div V1 \div T$

NP activity (nmol/min/mL) = $125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank})$

### 4. Calculation by Cell Count

Unit definition: At 30°C, for every 10^4 cells catalyzing hydrolysis per minute to produce 1 nmol tyrosine, this is defined as 1 enzyme activity unit.

NP activity (nmol/min/10^4 cells) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div (number\ of\ cells \times V1 \div V2) \div T$

NP activity (nmol/min/10^4 cells) = $125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \div cell\ count$

### Formula Parameters

| Parameter | Description | Value |
|---|---|---|
| $C_{standard}$ | Standard concentration | 0.25 μmol/mL |
| $V_{total\ reaction}$ | Total volume of the enzymatic reaction | 0.1 mL |

| | | |
|---|---|---|
| Cpr | Protein concentration of the crude enzyme solution | mg/mL |
| V1 | Volume of crude enzyme solution added to the reaction system | 0.02 mL |
| V2 | Total volume of the extract | 1 mL |
| T | Catalytic reaction time | 10 min |
| W | Sample mass | g |

## Precautions

- This 100T product can test 48 samples.
- Prepare reagents before use. After preparation, store at 4°C for up to 3 days.