Neutral Protease(NP) Activity Assay Kit

Product Code: 112036

Neutral Protease (NP) Activity Assay Kit - Microplate Method

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This kit measures neutral protease activity. Under neutral conditions, neutral protease catalyzes the hydrolysis of casein to produce tyrosine. Under alkaline conditions, tyrosine reduces phosphomolybdic acid compounds to form tungsten blue, which has a characteristic absorbance peak at 680 nm.

> Actual readings may vary depending on testing conditions and instruments.

Product Packing List

Item Code	Component	Quantity	Storage
112036.1	Reagent I	120 mL	2-8°C
112036.2	Reagent II	261.6 mg	2-8°C
112036.3	Reagent III	10 mg	Protect from light, 2-8°C
112036.4	Reagent IV	0.848 g	2-8°C
112036.5	Reagent V	4 mL	Protect from light, 2-8°C
112036.6	Standard	1 mL	Protect from light, -20°C
112036.m	Manual	1 copy	/

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Reagent V	--	--
Standard	--	--

Shipping and Storage

- **Shipping:** This product is shipped with ice packs.
- **Storage:** Store each component according to the listed conditions.
- **Shelf life:** 180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

1. **Tissue:** Add Reagent I according to a tissue mass (g) to Reagent I volume (mL) ratio of 1:5-10. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Reagent I. Homogenize in an ice bath, centrifuge at 8000g and 4°C for 10 min, and collect the supernatant as the crude enzyme extract.
2. **Bacteria and fungi:** Add Reagent I according to a cell count (10^4cells) to Reagent I volume (mL) ratio of 500-1000:1. It is recommended to use 500 × 10^4cells and add 1 mL Reagent I. Disrupt cells ultrasonically in an ice bath at 300 W, sonicate for 3 seconds with a 7-second interval, for a total time of 3 min. Centrifuge at 8000g and 4°C for 10 min, collect the supernatant, and keep it on ice for testing.
3. **Serum or culture medium:** Measure directly.

2. Reagent Preparation

1. Before use, add 4 mL distilled water to Reagent II and dissolve.
2. Before use, add 10 mL Reagent I to Reagent III. Dissolve by magnetic stirring in a boiling water bath. A layer of plastic wrap may be placed over the beaker to prevent water evaporation. Heating generally takes 15-30 min. This reagent is supersaturated; after thorough mixing, any remaining insoluble particles do not affect use.
3. Before use, add 20 mL distilled water to Reagent IV and dissolve.

3. Assay Procedure

1. Preheat the 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. Add samples and reagents according to the table below.

Component	Blank Tube	Standard Tube	Control Tube	Assay Tube
Crude enzyme solution	-	-	20 μL	20 μL
Reagent II	-	-	40 μL	-
Reagent III	-	-	-	40 μL
Incubate in a 30°C water bath for 10 min.				
Reagent II	-	-	-	40 μL
Reagent III	-	-	40 μL	-
Mix well, centrifuge at 4°C and 8000g for 10 min, then collect the supernatant.				
Supernatant	-	-	40 μL	40 μL
Distilled water	40 μL	-	-	-
Standard	-	40 μL	-	-
Reagent IV	200 μL	200 μL	200 μL	200 μL
Reagent V	40 μL	40 μL	40 μL	40 μL

Mix thoroughly, incubate in a 30°C water bath for 20 min, take 1 mL into a glass cuvette, and measure the absorbance at 680 nm. Record the values as A_{blank}, $A_{standard}$, $A_{control}$, and A_{assay}. The blank tube and standard tube only need to be measured 1-2 times.

Calculation of Neutral Protease Activity

1. Calculation Based on Sample Protein Concentration

Unit definition: At 30°C, the amount of enzyme that catalyzes hydrolysis to produce 1 nmol tyrosine per milligram of protein per minute is 1 enzyme activity unit.

NP activity (nmol/min/mg prot) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div (Cpr \times V1) \div T = 125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \div Cpr$

2. Calculation Based on Sample Mass

Unit definition: At 30°C, the amount of enzyme per gram of sample per minute that catalyzes hydrolysis to produce 1 nmol tyrosine is 1 enzyme activity unit.

NP activity (nmol/min/g, fresh weight) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div (W \times V1 \div V2) \div T = 125 \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \div W$

3. Calculation Based on Liquid Volume

Unit definition: At 30°C, the amount of enzyme per milliliter of sample per minute that catalyzes hydrolysis to produce 1 nmol tyrosine is 1 enzyme activity unit.

NP activity (nmol/min/mL) = $C_{standard} \times (A_{assay} - A_{control}) \div (A_{standard} - A_{blank}) \times V_{total\ reaction} \div V1 \div T = 125 \times (A_{measurement} - A_{control}) \div (A_{standard} - A_{blank})$

4. Calculation Based on Cell Count

Unit definition: At 30°C, the amount of enzyme per 10^4cells that catalyzes hydrolysis to produce 1 nmol tyrosine is 1 enzyme activity unit.

NP activity (nmol/min/10^4cells) = $C_{standard}$× ($A_{measurement}$- $A_{control}$) ÷ ($A_{standard}$- A_{blank}) × $V_{total\ reaction}$÷ (cell count × V1 ÷ V2) ÷ T = 125 × ($A_{measurement}$- $A_{control}$) ÷ ($A_{standard}$- A_{blank}) ÷ cell count

Formula Parameters

$C_{standard}$	250 nmol/mL standard tyrosine solution
$V_{total\ reaction}$	Total volume of enzymatic reaction, 0.1 mL
Cpr	Protein concentration of crude enzyme solution, mg/mL
V1	Volume of crude enzyme solution added to the reaction system, 0.02 mL
V2	Total volume of extract, 1 mL
T	Catalytic reaction time, 10 min
W	Sample mass, g

Precautions

1. After preparing reagents for immediate use, store them at 4°C and use within 3 days.
2. Because one control tube must be set up for each assay tube, this 100T kit can test 48 samples.
3. Required instruments and supplies to be prepared by the user include a mortar or homogenizer, benchtop centrifuge, microplate reader, water bath or metal bath, magnetic stirrer, adjustable pipettes, 96-well plate, ice, and distilled water.
4. If the reaction is weak and the A_{assay}- $A_{control}$difference is small, the reaction time, meaning the water bath time in the first step, may be appropriately extended to 20-30 min. The formula should be modified accordingly when calculating enzyme activity.