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CheKine™ Micro Neutral Protease (NP) Activity Assay Kit

Cat #: KTB2290 Size: 48 T/24 S 96 T/48 S

	Micro Neutral Protease (NP) Activity Assay Kit		
REF	**Cat #:** KTB2290	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Neutral Protease (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. CheKine™ Micro Neutral Protease (NP) Activity Assay Kit can be used to detect biological samples such as animal tissues, serum or plasma. In the kit, in neutral condition, NP can catalyze the hydrolysis of casein to produce tyrosine; in alkaline condition, tyrosine reduces phosphomolybdic acid compound to tungsten blue which has characteristic absorption peak at 680 nm, and the activity of NP is calculated by measuring its absorbance increase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	65 mL	65 mL×2	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅴ	2.4 mL	4.8 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 680 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge, thermostat
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅱ: Prepared before use. 48 T add 2.4 mL deionized water, 96 T add 4.8 mL deionized water to fully dissolve; The unused reagent can be stored at 4℃, protected from light for 3 days.

Working Reagent Ⅲ: Prepared before use. 48 T add 5 mL Reagent Ⅰ, 96 T add 10 mL Reagent Ⅰ, and dissolve by magnetic stirring in boiling water bath. (You can cover the beaker with a layer of fresh-keeping film, pay attention to observation, avoid all evaporation of water, generally heat for 15-30 min, the reagent is supersaturated, and the use of insoluble particles will not be affected after full mixing.) The unused reagent can be stored at 4℃, protected from light for 3 days.

Working Reagent Ⅳ: Prepared before use. 48 T add 12 mL deionized water, 96 T add 24 mL deionized water to fully dissolve; The unused reagent can be stored at 4℃, protected from light for 3 days.

Reagent Ⅴ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Note: Reagent Ⅱ has a pungent odor, Reagent Ⅴ is toxic, so it is recommended to experiment in a fume hood.

Standard： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, that is the crude enzyme solution, and place it on ice to be tested.
2. Plasma, Serum or other Liquid samples: Direct detection.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 680 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Working Reagent Ⅱ、Ⅲ、Ⅳ were kept in 30℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Sample	0	0	20	20
Working Reagent Ⅱ			0	40
Working Reagent Ⅲ			40	0
			Mix thoroughly, put in 30℃ for 10 min	
Working Reagent Ⅱ			40	0
Working Reagent Ⅲ			0	40
			After mixing, centrifuge at 8,000 g for 10 min at 4℃, take the supernatant	
Supernatant	0	0	40	40
Standard	0	40	0	0

Deionized water	40	0	0	0
Working Reagent Ⅳ	200	200	200	200
Reagent Ⅴ	40	40	40	40
Mix thoroughly, put in 30℃ for 20 min				

4. Add 200 μL to micro glass cuvette/96 well flat-bottom plate, detect the absorbance at 680 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, and the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: (1) The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. (2) If reaction is weak, ΔA_{Test} is less than 0.002, increase the sample quantity appropriately, or prolong the water bath time of the first step (20-30 min), and the formula should be modified when calculating the enzyme activity. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of the NP activity

(1) Calculated by sample 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 min at 30℃ every mg protein.

$NP(U/mg\ prot)= C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(Cpr\times V_{Sapmle})\div T=\mathbf{125\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

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 min at 30℃ every g sample.

$NP(U/g\ fresh\ weight)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div(W\times V_{Sapmle}\div V_{Extraction})\div T=\mathbf{125\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculated by volume of liquid samples

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 min at 30℃ every mL sample.

$NP(U/mL)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div V_{Sapmle}\div T=\mathbf{125\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: Standard tyrosine solution, 0.25 μmol/mL=250 nmol/mL; $V_{Total\ volume}$: Reaction total volume, 0.1 mL; Cpr: Sample protein concentration, mg/mL; V_{Sapmle}: The volume of crude enzyme was added to the reaction system, 0.02 mL; $V_{Extraction}$: Total volume of extractive liquid; T: The reaction time, 10 min; W: Sample weight, g.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

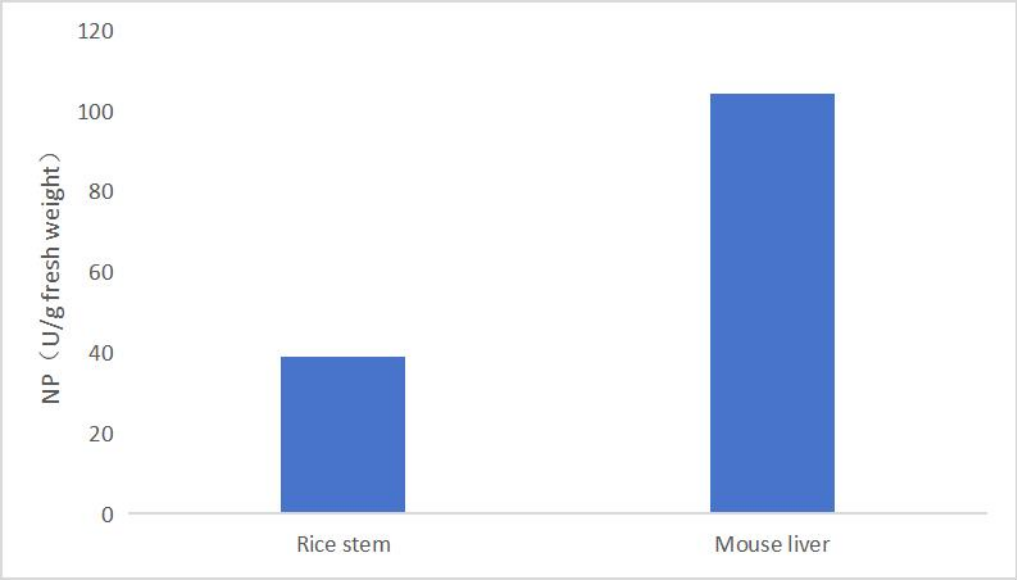

Figure 1. Determination of NP activity in rice stem and mouse liver by this kit.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1110	CheKine™ Lactate Dehydrogenase (LDH) Activity Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit
KTB2270	CheKine™ Micro Acid Protease (ACP) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.