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CheKine™ Micro NADP Phosphatase (NADPase) Activity Assay Kit

Cat #: KTB1017 Size: 48 T/96 T

	Micro NADP Phosphatase (NADPase) Activity Assay Kit		
REF	**Cat #:** KTB1017	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

NADP phosphatase (NADPase) is mainly present in plant tissues and is the only enzyme in organisms that catalyzes the degradation of $NADP^+$ to NAD^+. Together with NADK, it regulates the balance between NAD and NADP. NADPase can catalyze the hydrolysis of $NADP^+$ to NAD^+ and inorganic phosphorus, and its activity is determined by measuring the amount of inorganic phosphorus.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	7.5 mL	15 mL	4℃
Reagent Ⅱ	1×2	1×4	-20℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1	4℃, protected from light
Reagent Ⅴ	12.5 mL	25 mL	RT
Standard	1 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, incubator
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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

Reagent II: Prepared before use. Take one vial and add 1 mL of Reagent I to dissolve thoroughly for later use (each vial can be used for 25 T. To extend the usage time, two vials were prepared for 48 T and four vials were prepared for 96 T, ready for use).

Reagent III: Prepared before use. 48 T add 12.5 mL of deionized water, 96 T add 25 mL of deionized water, fully dissolve and set aside for use. The prepared reagent can be stored at 4℃, protected from light for 1 week.

Reagent IV: Prepared before use. 48 T add 12.5 mL of deionized water, 96 T add 25 mL of deionized water, fully dissolve and set aside for use. If it is difficult to dissolve, a water bath at 37℃ for 10 min can promote dissolution. The prepared reagent can be stored at 4℃, protected from light for 1 week.

Reagent V: Ready to use as supplied. Equilibrate to room temperature before use. Store at room temperature.

Standard: 10 μmol/mL standard phosphorus reserve solution, ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

0.5 μmol/mL standard phosphorus application solution preparation: Take 50 μL Standard added to 950 μL deionized water, thoroughly mix well. Store at 4℃.

Working Reagent： Prepare according to the ratio of deionized water: Reagent III: Reagent IV: Reagent V=2:1:1:1, and prepare according to the experimental dosage. The prepared Working Reagent should be light yellow. If it is colorless, the reagent will be ineffective, and if it is blue, it will be phosphorus contamination.

Notes: To avoid phosphorus pollution, it is necessary to ensure that tools such as beakers and pipettes are clean enough when preparing reagents. It is best to use new beakers, glass rods, and pipettes, or disposable plastic containers.

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.

Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)
Reagent I	120	120
Reagent II	40	40
Preheat at 37℃ (for mammals) or 25℃ (for other species) for 5 min		
Sample supernatant	40	
Deionized water		40

After accurately reacting at 37℃ (for mammals) or 25℃ (for other species) for 30 min, take a 95℃ water bath for 5 min (cover tightly to prevent water loss), cool, centrifuge at 25℃ for 5 min at 10,000 g, and take the supernatant.

3. Determination of phosphorus (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)	Blank Well (μL)

0.5 μmol/mL Standard	0	0	20	0
Deionized water	0	0	0	20
supernatant	20	20	0	0
Working Reagent	200	200	200	200

Mix well, let it stand at 25℃ for 30 min, and measure the absorbance value at 660 nm. The absorbance of test well, control well, standard well and blank well were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Control well, standard well and blank well only need to be done once or twice. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment.

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.

1. Calculated by protein concentration

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by the breakdown of NADP by NADase in every mg of tissue protein per h is one unit of NADase activity.

NADPase (U/mg prot)=$(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div A_{Standard}\div(V_{Sample}\times Cpr)\div T$**=$5\times\Delta A_{Test}\div A_{Standard}\div Cpr$**

2. Calculated by sample fresh weight

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by the breakdown of NADP by NADase in every g of tissue per h is one unit of NADase activity.

NADPase (U/g fresh weight)=$(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div A_{Standard}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=$5\times\Delta A_{Test}\div A_{Standard}\div W$**

$C_{Standard}$: 0.5 μmol/mL standard phosphorus application solution; V_{Total}: total volume of enzymatic reaction, 0.2 mL; V_{Sample}: sample volume added, 0.04 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 0.5 h; W: sample weight, g.

Typical Data

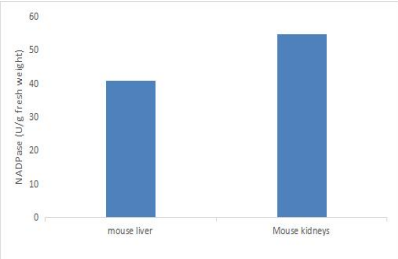

Figure 1. Determination NADPase activity in mouse liver and mouse kidneys by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.