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CheKine™ Micro Nitrite Reductase (NiR) Activity Assay Kit

Cat #: KTB4017 Size: 48 T/96 T

	Micro Nitrite Reductase (NiR) Activity Assay Kit		
REF	**Cat #:** KTB4017	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues, Bacteria, Fungi		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Nitrite reductase (NiR) is a crucial enzyme in the reduction process of nitrite nitrogen and plays a vital role in the nitrogen cycle of nature. It is widely distributed in microorganisms and plants, capable of catalyzing the reduction of nitrite, thereby decreasing the accumulation of nitrite nitrogen in the environment and alleviating its toxic effects on the growth and development of organisms. CheKine™ Micro Nitrite Reductase (NiR) Activity Assay Kit is designed to quantify nitrite reductase activity in plant tissues, bacterial, and fungal samples. It operates on the principle that nitrite reductase catalyzes the reduction of NO^{2-} to NO, thereby reducing the amount of NO^{2-} available in the sample to participate in diazotization reactions that form a purple-colored compound. The change in absorbance at 540 nm reflects the activity of NiR in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	56 mL	112 mL	4℃
Reagent Ⅰ	2 mL	4 mL	4℃
Reagent Ⅱ	1	1	4℃
Reagent Ⅲ	2.5 mL	5 mL	4℃
Reagent Ⅳ	5 mL	10 mL	4℃, protected from light
Reagent Ⅴ	5 mL	10 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

- Thermostatic water bath, ice maker, low-temperature centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

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. add 2.5 mL deionized water for 48 T and 5 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃ for 2 weeks.

Reagent Ⅲ： Ready to use as supplied. Equilibrate to room temperature before use (If precipitation occurs, it can be dissolved by heating at 70-80℃). Store at 4℃.

Reagent Ⅳ： Ready to use as supplied. Equilibrate to room temperature before use (If precipitation occurs, it can be dissolved by heating at 70-80℃). Store at 4℃, protected from light.

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

Working Reagent： Just before use, mix Reagent Ⅳ and Reagent Ⅴ in a 1: 1 ratio according to the required volume, preparing the mixture freshly as needed.

Standard： 10 μmol/mL sodium nitrite standard solution. Store at 4℃.

Standard preparation: Using 10 μmol/mL sodium nitrite standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (μmol/mL)
Std.1	20 μL 10 μmol/mL Standard	980	0.2
Std.2	500 μL of Std.1 (0.2 μmol/mL)	500	0.1
Std.3	500 μL of Std.2 (0.1 μmol/mL)	500	0.05
Std.4	500 μL of Std.3 (0.05 μmol/mL)	500	0.025
Std.5	500 μL of Std.4 (0.025 μmol/mL)	500	0.0125
Std.6	500 μL of Std.5 (0.0125 μmol/mL)	500	0.00625
Std.7	500 μL of Std.6 (0.00625 μmol/mL)	500	0.003125
Std.8	0	500	0（Blank Tube）

Note: Std.8 refers to the Blank Tube.

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. Tissue samples: Weigh approximately 0.1 g of fresh tissue and add 1 mL of Extraction Buffer. Crush the tissue in the buffer and then proceed with ultrasonic extraction in an ice bath (power 300 W, ultrasonic 5 s, interval 8 s, total time is 5 min). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Bacterial or fungal cell: Collect 5×10^6 bacterial or fungal cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacterial or fungal cells 3 min (power 300 W, ultrasonic 3 s, interval 7 s, total time is 3 min). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

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 540 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are carried out in a 1.5 mL EP tube.):

Reagent	Matrix Tube（μL）	Control Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Sample	0	20	20	0
Deionized water	20	40	0	0
Reagent Ⅰ	40	0	40	0
Reagent Ⅱ	40	40	40	0
After mixing, incubate at 25℃ for 1 h				
Reagent Ⅲ	40	40	40	0
Shake vigorously for 30 s, then let it stand at room temperature for 5 min. Afterwards, transfer the supernatant to a micro glass cuvette or a 96-well plate.				
Supernatant	70	70	70	0
Standard	0	0	0	70
Working Reagent	140	140	140	140
Thoroughly mix and then let it stand at room temperature for 5 min. Measure the absorbance at 540 nm for each tube, recording the values as A_{Matrix}, $A_{Control}$, A_{Test}, $A_{Standard}$ and A_{Blank}, respectively. Calculate $\Delta A_{Test}=A_{Matrix}-(A_{Test}-A_{Control})$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$。				

Note: Standard curve, Blank Tube and Matrix Tube 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. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If A_{Test} is greater than 0.7, the sample supernatant can be further diluted by Extraction Buffer, and the calculation result should be multiplied by the dilution multiple, or reduce the sample size for extraction.

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. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of NIR activity:

(1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NO^{2-} per mg of tissue protein per h.

$NiR(U/mg\ prot)=x\times V_{Total}\div V_{Sample}\times V_{Total\ Sample}\div(Cpr\times V_{Total\ Sample})\div T$**$=x\times 7\div Cpr$**

(2) Calculated by sample fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NO^{2-} per g of tissue per h.

$NiR(U/g\ weight)=x\times V_{Total}\div V_{Sample}\times V_{Total\ Sample}\div W\div T$**$=x\times 7\div W$**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NO^{2-} per 10^4 of bacterial or fungal cells per h.

NiR(U/g weight)=x×V_{Total}÷V_{Sample}×$V_{Total\ Sample}$÷N÷T=**x×7÷N**

V_{Total}: the reaction system volume before taking the supernatant, 0.14 mL; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 1 h; Cpr: sample protein concentration, mg/mL; W: sample weight, g, N: total number of bacterial or fungal cells, 10^4.

Typical Data

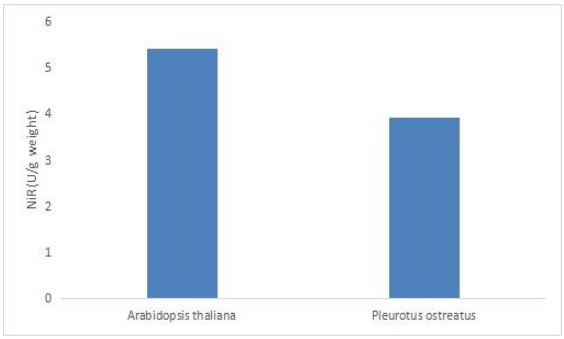

Figure 1. NiR activity in Arabidopsis thaliana and Pleurotus ostreatus was detected with this kit

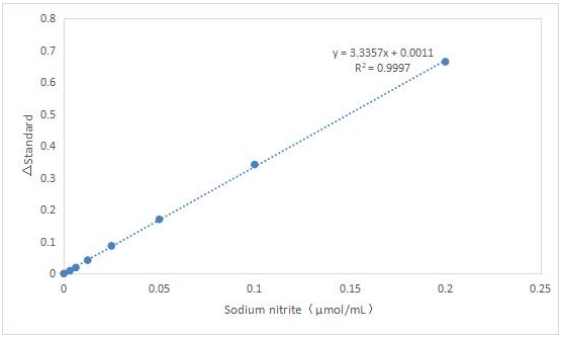

Figure 2. Standard Curve of NiR assay

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.