Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Soil Nitrite Reductase (S-NiR) Activity Assay Kit

Cat #: KTB4036 Size: 48 T/48 S 96 T/96 S

	Micro Nitrite Reductase (NiR) Activity Assay Kit		
REF	**Cat #:** KTB4036	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil Sample		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Soil Nitrite Reductase: Soil nitrite reductase is one of the key enzymes involved in denitrification, catalyzing the reduction of nitrite (NO^{2-}) to nitric oxide (NO). Its activity reflects the efficiency of nitrogen transformation during biological degradation processes and provides valuable insights into the patterns of nitrogen conversion. CheKine™ Micro Soil Nitrite Reductase (S-NiR) Activity Assay Kit is designed to quantify nitrite reductase activity in soil samples. The assay principle is based on the enzyme's ability to reduce NO^{2-} to NO, which decreases the concentration of NO^{2-} available for diazotization reactions that produce a purple-red compound. The change in absorbance at 540 nm directly correlates with the activity of nitrite reductase in the soil sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	3 mL	6 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅲ	5.5 mL	11 mL	4℃
Reagent Ⅳ	10 mL	20 mL	4℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	1 mL	1 mL	4℃

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 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Oven, 30-50 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water

Reagent Preparation

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

Working Reagent Ⅱ： Prepared before use. Add 5.5 mL deionized water for 48 T and 11 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.

Working Reagent Ⅴ： Prepared before use. Add 10 mL deionized water for 48 T and 20 mL deionized water for 96 T to fully dissolve. Store at 4℃, protected from light, for up to one week.

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

Working Reagent： Prepared before use. Mix Reagent Ⅳ and Working 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	100 μL 10 μmol/mL Standard	900	1
Std.2	160 μL of Std.1 (1 μmol/mL)	40	0.8
Std.3	120 μL of Std.1 (1 μmol/mL)	80	0.6
Std.4	80 μL of Std.1 (1 μmol/mL)	120	0.4
Std.5	40 μL of Std.1 (1 μmol/mL)	160	0.2
Std.6	20 μL of Std.1 (1 μmol/mL)	180	0.1
Std.7	10 μL of Std.1 (1 μmol/mL)	190	0.05
Std.8	0	200	0（Blank Tube）

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended.

Fresh soil samples should be air-dried naturally or dried in an oven at 37℃, then passed through a 30-50 mesh sieve.

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)
Air-dried soil sample (mg)	0	40	40	0
Deionized water	0	40	0	0

Reagent Ⅰ	40	0	40	0
Working Reagent Ⅱ	40	40	40	0
After mixing, incubate at 25℃ for 3 h				
Reagent Ⅲ	40	40	40	0
Shake vigorously for 30 s, centrifuge at 10,000 rpm for 10 min at 4℃. 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, 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 2.0, the sample supernatant can be further diluted by deionized water, 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, and substitute the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of S-NiR activity:

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 soil sample per d.

S-NiR(U/g soil)=$x\times V_{Total\ reaction}\div W\div T$**=0.96×x÷W**

V_{Total}: the reaction system volume, 0.12 mL; W: Sample mass, 0.04 g, T: Reaction time, 3 h=1/8 d.

Typical Data

Typical standard curve:

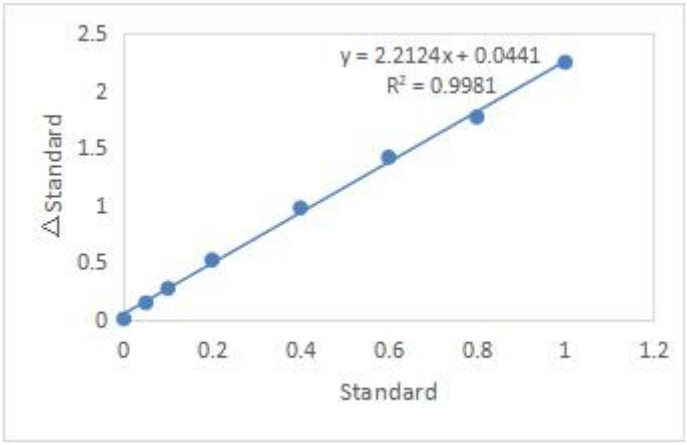

Figure1. Standard Curve for $NaNO_2$

Examples:

Take 0.04 g of fresh soil sample that has been dried in a 37 ℃ oven and use 96-well plate to calculate ΔA_{Test}=1.266-(1.082-0.052)=0.236, x=0.087. The content calculated according to the soil sample mass is as follows:

S-NiR(U/g soil)=0.96×0.087÷0.04=2.088 U/g.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) 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.