



CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit

Cat #: KTB4010

Size: 48 T/48 S 96 T/96 S

	Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit		
REF	Cat #: KTB4010	LOT	Lot #: Refer to product label
	Applicable: Soil		
	Storage: Stored at -20°C for 12 months, protected from light		

Assay Principle

Soil Nitrate Reductase (S-NR) catalyzes the reduction of nitrate to nitrite, $\text{NO}_3^- + \text{NADH} + \text{H}^+ \rightarrow \text{NO}_2^- + \text{NAD}^+ + \text{H}_2\text{O}$; The resulting nitrite can react with P-Sulfanilic Acid and α -Naphthylamine under acidic conditions and generate azo compounds with red color, which has maximum absorbance peak at 540 nm and can be determined by spectrophotometry.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	15 mL	30 mL	-20°C
Reagent II	Powder×1 vial	Powder×1 vial	-20°C
Reagent III	11 mL	22 mL	4°C
Reagent IV	11 mL	22 mL	4°C, protected from light
Standard	1 mL	2 mL	-20°C

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
- Temperature controlled oscillator, centrifuge, adjustable water bath
- Deionized water, PBS

Reagent Preparation

Reagent I : Ready to use as supplied. Place on ice throughout use. Store it separately at -20°C. The remaining reagent can also be

stored at -20°C for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent II :Prepare before use, add 10 mL of PBS for 48 T, add 20 mL of PBS for 96 T, and fully dissolve before use, place on ice throughout use. The remaining reagent can also be stored at -20°C for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C (If crystallization occurs, dissolve in a water bath at 60°C-90°C before use).

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Standard: Prepare before use, Take 0.1 mL standard and add deionized water to a final concentration at 0.1 µmol/mL, which is fresh prepared. The remaining Standard can also be stored at -20°C for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Naturally dried fresh soil samples or air drying in oven at 37°C, pass 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 performed in 1.5 mL tubes):

Reagent	Test Well (µL)	Control Well (µL)	Standard Well (µL)	Blank Well (µL)
Air dried soil sample (mg)	60	60	0	0
Standard	0	0	60	0
Deionized Water	0	225	0	285
Reagent I	225	0	225	0
Reagent II	75	75	75	75
Mix well, shake at 37°C for 24 h, then 8,000 g, centrifuge for 10 min at 25°C, and keep the supernatant for test				
Supernatant	130	130	130	130
Reagent III	85	85	85	85
Reagent IV	85	85	85	85

3. Mix well and keep for 20 min at RT. 4,000 g, centrifuge at 25°C for 10 min, take 200 µL supernatant to a microglass cuvette or 96 well plate, and measure the absorbance at 540 nm. The absorbance of blank well, standard well, test well, control well were recorded as A_{Blank} , $A_{Standard}$, A_{Test} and $A_{Control}$. Finally, calculate $\Delta A_{Test} = A_{Test} - A_{Control}$, $\Delta A_{Standard} = A_{Standard} - A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. Control well is necessary for each test. In order to guarantee the accuracy of experimental results, pre-experiments are suggested for 2-3 samples with potential significant difference. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.2, 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.

Definition of unit: 1 µmol NO₂⁻ produced by per gram soil within one day equals one unit of S-NR activity.

S-NR (U/g soil sample) = $C_{Standard} \times \Delta A_{Test} \div \Delta A_{Standard} \times V_{Total} \div W \div T = \mathbf{0.5 \times \Delta A_{Test} \div \Delta A_{Standard}}$

C_{Standard}: standard concentration, 0.1 μmol/mL; V_{Total}: total volume of the reaction system, 0.3 mL; T: reaction time, 1 d; W: sample weight, 0.06 g.

Typical Data

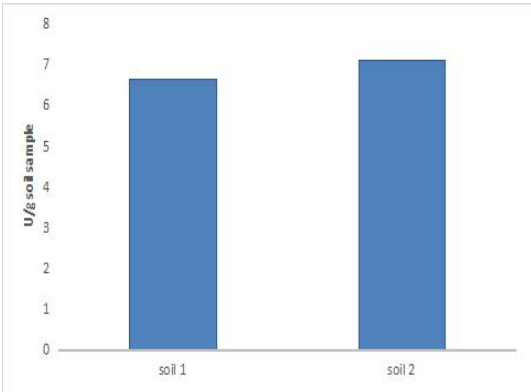


Figure 1. The activity of nitrate reductase in soil

Recommended Products

Catalog No.	Product Name
KTB3040	CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase (GDH) 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.