

EZMeta™ Soil Nitrate Reductase (S-NR) Colorimetric Activity Kit

Cat #: D-AKC4010

Size: 48T / 96T

Storage: Stored at -20°C for 12 months, protected from light

Product Information

Applicable samples: Soil

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 | 12 mL | 24 mL | -20°C |
| Reagent II | 1 | 1 | -20°C |
| Reagent III | 9 mL | 18 mL | 4°C |
| Reagent IV | 9 mL | 18 mL | 4°C, protected from light |
| Standard | 1 mL | 2 mL | -20°C |

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. Equilibrate to room temperature before use. Store it separately at -20°C. Repeated freezing and thawing are prohibited.

Reagent II : Prepare before use, add 8 mL of PBS for 48 T, add 16 mL of PBS for 96 T, and fully dissolve before use. Store it separately at -20°C. Repeated freezing and thawing are prohibited.

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: Take 0.1 mL standard and add deionized water to a final concentration at 0.1 µmol/mL. Store it separately at -20°C. Repeated freezing and thawing are prohibited.

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 |
| ReagentIII | 85 | 85 | 85 | 85 |
| ReagentIV | 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 $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

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.

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.

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

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

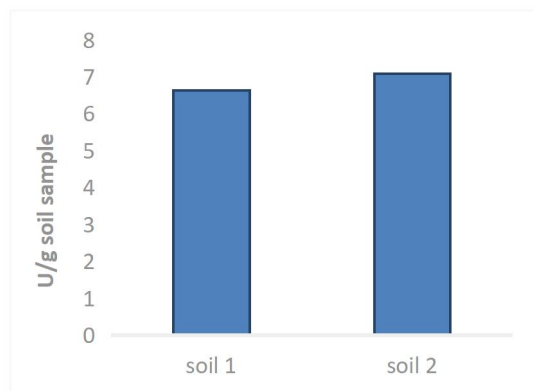


Figure 1. The activity of nitrate reductase in soil

Disclaimer

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