EZMeta™ Water and Soil Nitrite Content Colorimetric Assay Kit

Cat #: D-AKC3050

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Water, Soil

Assay Principle

Nitrite exists widely in water and soil, which is not only an important intermediate of organic nitrogen decomposition, but also may come from pollution. Excessive intake of the human body can induce canceration of the digestive system. EZMeta™ Water and Soil Nitrite Content Colorimetric Assay Kit provides a simple, convenient and rapid method for the determination of nitrite content, which is suitable for water and soil samples. The detection principle is that under acidic conditions, nitrite reacts with p-aminobenzene sulfonic acid to form diazo compounds, and then reacts with Nmuri 1-naphthyl ethylenediamine to form purplish red azo compounds with a characteristic absorption peak at 540 nm.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent Ⅰ	100 mL	4°C
Reagent Ⅱ	10 mL	4°C, protected from light
Reagent Ⅲ	10 mL	4°C, protected from light
$NaNO_2$ Standard	500 μL	4°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

·Ice maker, centrifuge, 2 mm sieve mesh

·Deionized water

Reagent Preparation

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

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

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

Standard Curve Setting: 1 mmol/mL Standard was diluted to 10 μmol/mL with Reagent I. Dilute the 10 μmol/mL Standard with Reagent I to 5, 2.5, 1.25, 0.625, 0.313, 0.156 μmoL/mL as indicated in the table below.

Num.	Volume of Standard	Volume of Reagent Ⅰ (μL)	The Concentration of Standard (μmol/mL)
Std.1	200 μL of Standard	0	10
Std.2	100 μL of Std.1 (10 μmol/mL)	100	5
Std.3	100 μL of Std.2 (5 μmol/mL)	100	2.5
Std.4	100 μL of Std.3 (2.5 μmol/mL)	100	1.25
Std.5	100 μL of Std.4 (1.25 μmol/mL)	100	0.625
Std.6	100 μL of Std.5 (0.625 μmol/mL)	100	0.313
Std.7	100 μL of Std.6 (0.313 μmol/mL)	100	0.156

Sample Preparation

1. Soil samples: Take appropriate amount of soil samples, remove stones, branches and other impurities, filter with 2mm sieve mesh, accurately weigh 0.5 g, add 1 mL Reagent Ⅰ, shake at room temperature for 1 h, 8,000g, centrifuge 15 min at 25°C, and take the supernatant to be tested.

2. Water sample: Direct detection. If it is turbid, it can be determined after centrifugation.

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. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Reagent Ⅰ	70	0	0
Standard	0	70	0
Sample	0	0	70
Reagent Ⅱ	65	65	65
ReagentⅢ	65	65	65

3. Mix well and then incubatefor 15 min at 25°C. Then reading the values at 540 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2.0, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor, or 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.

1. Drawing of standard curve

With the concentration of the Standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

Substitute the ΔA_{Test} into the equation to obtain the y value (μmoL/mL).

2. Calculate the content of NO^{2-} in sample

(1) Soil samples:

NO^{2-} (μmol/g)=$y \times V_{Sample} \div (W \times V_{Sample} \div V_{Total}) \times n$=**$2y \times n$**

(2) Water sample:

NO^{2-} (μmol/mL)=$y \div V_{Total} \times n$=**$y \times n$**

Where: V_{Sample}: Sample volume, 0.07 mL; W: sample weight, 0.5 g; V_{Total}: Add the volume of Reagent I, 1 mL; n: dilution factor.

Typical Data

Typical standard curve:

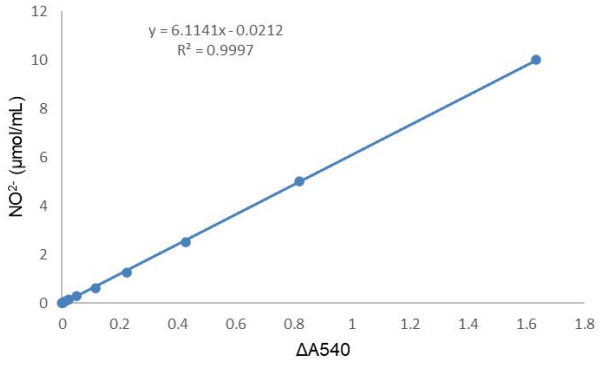

Figure1. Standard Curve for NO^{2-}.

Examples

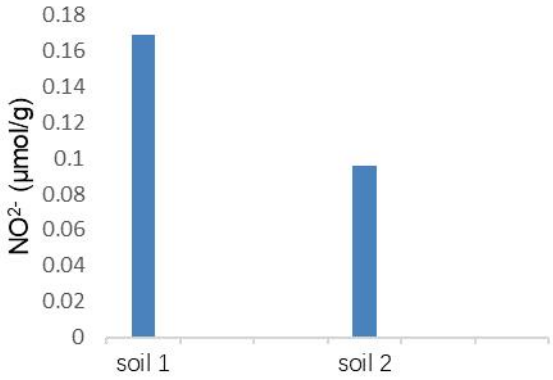

Figure 2. NO^{2-} content in soil. Assays were performed following kit protocol.

Disclaimer

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