

Food Nitrite Assay Kit (NEDD, Micro Method)

N1507975

Storage: See component table.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Product Introduction

In food, nitrite can bind to myoglobin in meat products to form stable complexes. It is used as a color-retention agent in the food industry to maintain the favorable color of meat products and inhibit the growth of *Clostridium botulinum*, thus improving the food safety of meat products. However, long-term intake of food with excessive nitrite may increase the risk of digestive system cancer.

Detection Principle:

Under acidic conditions, nitrite reacts with sulfanilic acid via diazotization to form diazonium salt. The diazonium salt further couples with N-1-naphthylethylenediamine to produce a magenta azo compound, which shows a characteristic absorption peak at 540 nm.

Assay range: 1.563-100 μM .

Sensitivity: 1.563 μM .

Kit Components

Storage Unit No.	Storage Unit Name	Component No.	Component Name	Appearance	96T	Storage	Quantity Per Test
N1507975NT	Food Nitrite Assay Kit (NEDD, Microplate Method) (RT Components)	N1507975A	Extraction Solution I	Colorless clear liquid	70 mL	Room temperature.	As needed
		N1507975B	Extraction Solution II	Yellow liquid	70 mL	Room temperature.	As needed
		N1507975C	Extraction Solution III	Colorless clear liquid	70 mL	Room temperature	As needed
		N1507975D	Activated Carbon	Black solid	120 mg	Room temperature.	As needed
N1507975WT	Food Nitrite Assay Kit (NEDD, Microplate Method) (2-8°C Components)	N1507975E	Reagent I	Colorless clear liquid	10 mL	2-8°C. Protect from light	65 μL
		N1507975F	Reagent II	Colorless clear liquid	10 mL	2-8°C. Protect from light	65 μL
		N1507975G	NaNO ₂ Standard (1M)	Colorless clear liquid	500 μL	2-8°C.	70 μL

Required instruments and reagents not provided

(1) Microplate reader or visible-spectrophotometer (capable of measuring absorbance at

- 540 nm);
- (2) 96-well plate or micro-glass cuvettes, adjustable pipettes and corresponding tips;
 - (3) Ice-maker, centrifuge, water-bath;
 - (4) Deionized water;
 - (5) Mortar.

Operating Procedures

1. Reagent Preparation

Reagent Name	Preparation	Notes
Extraction Solution I	Ready-to-use	Store at room temperature. This is a saturated solution; slight precipitation is normal. Mix thoroughly before use.
Extraction Solution II	Ready-to-use	Store at room temperature.
Extraction Solution III	Ready-to-use	Store at room temperature.
Activated Carbon	Ready-to-use	Store at room temperature.
Reagent I	Ready-to-use, equilibrate to room temperature before use	Store at 2-8 °C, protected from light.
Reagent II	Ready-to-use, equilibrate to room temperature before use	Store at 2-8 °C, protected from light.
NaNO ₂ Standard (1 mol/L)	-	Store at 2-8 °C

Note: Reagent II and NaNO₂ Standard (1 mol/L) are toxic. Perform all experimental operations in a fume hood with proper personal protection.

2. Calibration Curve Preparation

Dilute 1 mol/L NaNO₂ Standard with deionized water to prepare 100 µM working standard solution. As shown in the table below, serially dilute the 100 µM NaNO₂ working standard with deionized water to obtain serial standard solutions of 50, 25, 12.5, 6.25, 3.125 and 1.563 µM.

No.	Volume of Standard Solution	Volume of Deionized Water (µL)	Concentration (µM)
Std.1	200 µL of 100 µM NaNO ₂ Standard	0	100
Std.2	100 µL of Std.1 (100 µM)	100	50
Std.3	100 µL of Std.2 (50 µM)	100	25
Std.4	100 µL of Std.3 (25 µM)	100	12.5
Std.5	100 µL of Std.4 (12.5 µM)	100	6.25
Std.6	100 µL of Std.5 (6.25 µM)	100	3.125
Std.7	100 µL of Std.6 (3.125 µM)	100	1.563
Std.8	0	100	0

Note: The calibration curve shall be generated with standards in each independent experiment. The diluted standard working solution is unstable and must be used within 4 hours.

3. Sample Preparation

Weigh approximately 0.2 g fresh-weight (or 0.05 g dry-weight) sample and homogenize thoroughly. Add 0.5 mL Extraction Solution I, incubate in boiling-water bath for 15 min, then cool to room temperature. Add 0.5 mL Extraction Solution II and mix thoroughly by vortexing. Add 0.5 mL Extraction Solution III, followed by a small amount of activated carbon (~1 mg) using tweezers. Mix well and allow to stand for 30 min. Centrifuge at 8000 g, 25 °C for 15 min, collect the supernatant for subsequent measurement.

Note: The sample mixture will turn viscous after adding Extraction Solution II and III, which is normal.

4. Experimental Procedures

4.1 Pre-warm the microplate reader or visible spectrophotometer for 30 min, and set the detection wavelength to 540 nm. Zero the visible spectrophotometer with deionized water.

4.2 Operation (Perform the following operations in 96-well plate or micro-glass cuvettes, refer to the table below):

Reagent	Test Well (μL)	Standard Well (μL)	Blank Well (μL)
Sample Supernatant	70	0	0
NaNO ₂ working standard	0	70	0
Deionized Water	0	0	70
Reagent I	65	65	65
Reagent II	65	65	65

Mix well, incubate at 25 °C for 15 minutes, measure the absorbance at 540 nm, recorded as A_{Test} , A_{Standard} , and A_{Blank} . Calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$ and $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$. Calculate: $\Delta A_{\text{sample}} = A_{\text{sample}} - A_{\text{blank}}$, $\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$.

Note: The blank and standard wells need to be measured only once. It is recommended to perform a preliminary experiment with 2-3 samples showing expected large differences before formal testing. If ΔA_{Test} is less than 0.001, appropriately increase the sample amount. If ΔA_{Test} is greater than 1.0, further dilute the sample with deionized water and multiply the result by the dilution factor, or reduce the amount of sample used for extraction.

5. Result Calculation

Note: Both derived formula and simplified formula are provided in this manual, and they yield identical calculation results. It is recommended to use the bold simplified formula for final calculation.

5.1 Establishment of calibration curve

Plot the calibration curve with standard-solution concentration on the Y-axis and $\Delta A_{\text{standard}}$ on the X-axis. Substitute ΔA_{sample} into the regression equation of calibration curve to obtain the corresponding concentration y (μM) of sample.

5.2 Calculation of nitrite content in samples

$$\text{NO}_2^- (\mu\text{g/g}) = y \times V_{\text{Total}} \times 46 \div 1,000 \div W \times n = 0.069 \times y \div W \times n$$

Parameter Description:

V_{Total} : Total volume of the sample extract, 1.5 mL;

46: Molecular weight of NO_2^- , g/mol;

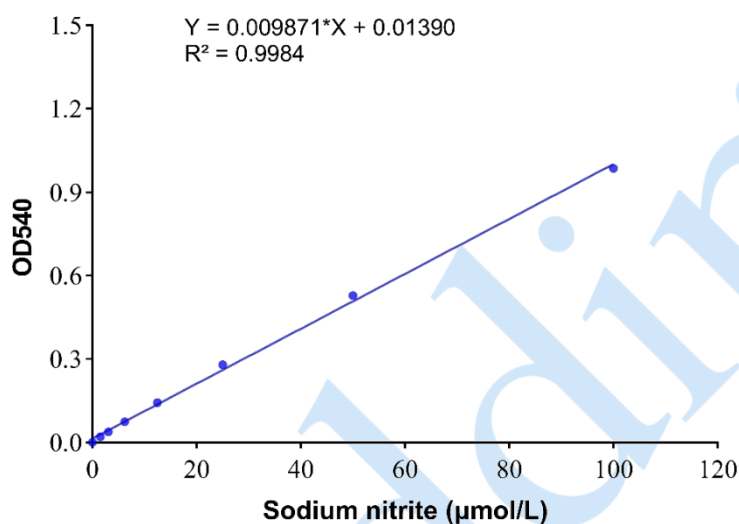
1,000: Unit conversion factor, 1 L = 1,000 mL;

W: Sample mass, g;

n: Dilution factor of the sample.

6. Example Results

Typical Standard Curve:



Experimental Examples:

For 0.2 g of pickled vegetable measured using a 96-well plate: $\Delta A = 0.103$. Substituting into the equation yields $y = 10.345 \mu\text{M}$. The calculated nitrite content is: $\text{NO}_2^- (\mu\text{g/g}) = 0.069 \times 10.345 \div 0.2 \times 1 = 3.569 \mu\text{g/g}$.

For 0.2 g of canned meat measured using a 96-well plate: $\Delta A = 0.007$. Substituting into the equation yields $y = 0.75 \mu\text{M}$. The calculated nitrite content is: $\text{NO}_2^- (\mu\text{g/g}) = 0.069 \times 0.75 \div 0.2 \times 1 = 0.259 \mu\text{g/g}$.

Notes

This product is for scientific research use only and is not intended for clinical diagnosis. For your safety and health, please wear a lab coat and disposable gloves during operation.