

Food Nitrite Assay Kit (NED, Colorimetric method)

N1521784

Storage: 2-8°C and Room temperature (See Table of Components for details.)

Shipped: ice packs and dry ice. Upon receipt, store immediately under specified storage conditions.

Product Introduction

Nitrites are widely used as color fixatives and preservatives in meat product processing. During processing, nitrites release nitric oxide, which binds with myoglobin in meat to form nitrosomyoglobin, maintaining the ruddy color of meat products. Meanwhile, they can significantly inhibit spoilage bacteria such as *Clostridium botulinum*, effectively eliminating the risk of botulinum toxin poisoning.

Nitrites pose definite health hazards: acute massive intake can induce acute methemoglobinemia. In the acidic gastric environment without blocking agents such as vitamin C, nitrites tend to react with secondary amines and amides in food to generate N-nitroso compounds, and long-term exposure increases the risk of digestive tract cancers.

This kit adopts the classic Griess spectrophotometric method stipulated in national standards. Under acidic conditions, nitrites undergo diazotization reaction with sulfanilic acid, followed by coupling reaction with N-(1-naphthyl) ethylenediamine dihydrochloride to form characteristic purplish-red azo dyes, which have maximum absorption at a wavelength of 540 nm. Within the linear range of 1.563-100 µM, the absorbance difference of the chromogenic system is proportional to nitrite concentration. Trace and accurate quantification of nitrites in food samples can be achieved via the standard curve method.

This kit is only for scientific research purposes and shall not be used for clinical diagnosis or other applications.

Kit Components

Storage Unit No.	Storage Unit Name	Component No.	Component Name	Appearance	50T	Storage	Quantity Per Test
N1521784NT	Food Nitrite Assay Kit(NED, Colorimetric method) (RT Components)	N1521784A	Extraction Solution I	Colorless clear liquid	50 mL	Room temperature.	As needed
		N1521784B	Extraction Solution II	Yellow liquid	50 mL	Room temperature.	As needed
		N1521784C	Extraction Solution III	Colorless clear liquid	50 mL	Room temperature	As needed
		N1521784D	Activated Carbon	Black solid	0.1 g	Room temperature.	As needed

Storage Unit No.	Storage Unit Name	Component No.	Component Name	Appearance	50T	Storage	Quantity Per Test
N1521784WT	Food Nitrite Assay Kit (NED, Colorimetric method) (2-8°C Components)	N1521784E	Reagent I	Colorless clear liquid	25 mL	2-8°C. Protect from light.	325 µL
		N1521784F	Reagent II	Colorless clear liquid	25 mL	2-8°C. Protect from light.	325 µL
		N1521784G	NaNO ₂ Standard (1M)	Colorless clear liquid	2.5 mL	2-8°C.	350 µL

Instructions for Use

1. Materials Preparation

Additional materials required:

- (1) Instruments: Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm.
- (2) Consumables: 96-well microplate, ice maker, centrifuge, water bath.

2. Reagent Composition and Storage

- (1) Extraction Solution I: Ready-to-use, store at room temperature. It is a saturated solution; slight precipitation is normal. Shake thoroughly before use.
- (2) Extraction Solution II: Ready-to-use, store at room temperature.
- (3) Extraction Solution III: Ready-to-use, store at room temperature.
- (4) Activated Carbon: Ready-to-use, store at room temperature.
- (5) Reagent I: Ready-to-use, equilibrate to room temperature before use; store at 4 °C protected from light.
- (6) Reagent II: Ready-to-use, equilibrate to room temperature before use; store at 4 °C protected from light.
- (7) Standard Curve Preparation
- (8) Dilute 1 M NaNO₂ Standard to 100 µM with deionized water. Serially dilute the 100 µM NaNO₂ Standard to 50, 25, 12.5, 6.25, 3.125 and 1.563 µM with deionized water as shown in the table below.

No.	Volume of Standard Solution	Volume of Deionized Water (µL)	Concentration (µM)
Std.1	800 µL of 100 µM NaNO ₂ Standard	0	100
Std.2	400 µL of Std.1 (100 µM)	400	50
Std.3	400 µL of Std.2 (50 µM)	400	25
Std.4	400 µL of Std.3 (25 µM)	400	12.5
Std.5	400 µL of Std.4 (12.5 µM)	400	6.25
Std.6	400 µL of Std.5 (6.25 µM)	400	3.125
Std.7	400 µL of Std.6 (3.125 µM)	400	1.563
Std.8	0	800	0

Note 1: Reagent II and NaNO_2 Standard are toxic. All dilutions and operations should be performed in a fume hood.

Note 2: A standard curve must be generated for each independent experiment. Diluted standard solutions are unstable and must be used within 4 hours after preparation.

3. Sample Pretreatment

Weigh approximately 0.2 g fresh sample or 0.05 g dry sample and fully homogenize. Add 0.5 mL Extraction Solution I, tightly cap the centrifuge tube and heat in a boiling water bath for 15 minutes, then cool to room temperature. Add 0.5 mL Extraction Solution II and vortex to mix thoroughly. Add another 0.5 mL Extraction Solution III, introduce approximately 1 mg activated carbon with tweezers and mix well. Keep the mixture protected from light at ambient temperature for 30 minutes. Centrifuge at $8000 \times g$ and 25°C for 15 minutes, then carefully collect the middle clear supernatant for subsequent detection.

Note 1: Tighten the tube cap during boiling water bath incubation to avoid solvent evaporation and deviation in total extraction volume.

Note 2: Viscosity increase after adding Extraction Solution II and Extraction Solution III is normal and will not affect decolorization or centrifugation. For deeply colored samples, the dosage of activated carbon can be increased to 2-3 mg, but avoid excessive addition which may adsorb nitrite and lead to underreported results. If the supernatant remains turbid after centrifugation, re-centrifuge under identical conditions for another 10 minutes before sampling.

4. Experimental Procedures

- (1) Preheat the microplate reader or visible spectrophotometer for 30 minutes and set the wavelength to 540 nm. Zero the visible spectrophotometer with deionized water.
- (2) Reaction System Setup (Operate in a 96-well microplate or micro quartz cuvette).

Reagent	Test Well (μL)	Standard Well (μL)	Blank Well (μL)
Sample Supernatant	350	0	0
NaNO_2 Standard	0	350	0
Deionized Water	0	0	350
Reagent I	325	325	325
Reagent II	325	325	325

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

Two equivalent formulas are provided below. The simplified bold formula is recommended for final calculation.

(1) Standard Curve Plotting

Plot the standard curve with nitrite concentration on the Y-axis and corrected absorbance ΔA_{std} on the X-axis. Substitute ΔA_{test} into the linear equation to solve for concentration y (unit: μM).

(2) Calculation of Nitrite Content in Samples

$$\text{NO}_2^- (\mu\text{g/g}) = y \times V_{\text{total}} \times 46 \div 1000 \div W \times n = 0.069 \times y \div W \times n$$

Parameter Explanation:

y : Nitrite concentration of sample extract calculated from standard curve, unit: μM ;

V_{total} : Total extraction volume = 1.5 mL;

46: Relative molecular mass of nitrite ion (NO_2^-), g/mol;

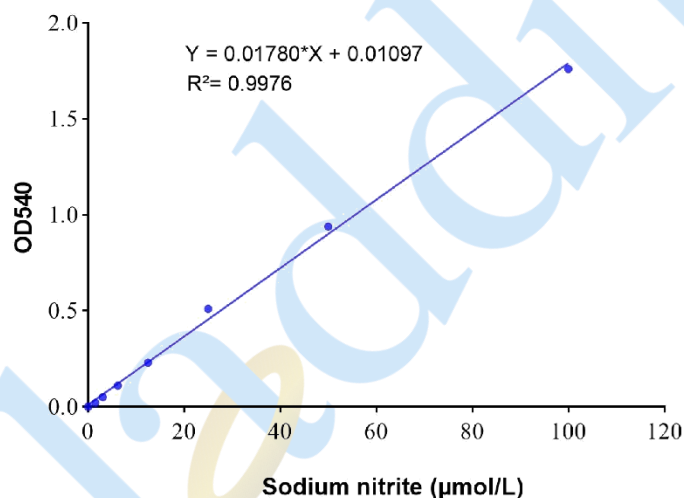
1000: Volume conversion factor (1 L = 1000 mL);

W : Weighed sample mass, unit: g;

n : Supernatant dilution factor ($n = 1$ if undiluted).

6. Example Results

Typical Standard Curve:



Note: This is a typical standard curve, actual curve parameters may vary slightly due to instrument and operation differences.

Notes

1. Kit reagents contain carcinogenic aromatic amines and toxic nitrites. All operations must be carried out in a fume hood. Wear a lab coat, nitrile gloves and safety goggles throughout the experiment, and avoid direct skin contact with any reagents.
2. The entire chromogenic incubation process must be protected from light to prevent photodegradation of azo dyes and resultant test deviation. If the sample absorbance value is

excessively high, reduce the sample weight or dilute the supernatant, and convert the dilution factor in the final calculation. For high-fat and heavily pigmented samples, remove matrix interference via activated carbon decolorization and n-hexane degreasing.

3. A preliminary test is recommended before formal detection using samples with markedly high and low concentrations to verify the extraction and chromogenic system. Do not mix kit components with reagents from other brands to avoid cross-interference and compromised accuracy.

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