



## CheKine™ Micro Food Nitrite Assay Kit

Cat #: KTB3051

Size: 96 T

	<b>Micro Food Nitrite Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB3051	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Food		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

### Assay Principle

In food, nitrite can combine with myoglobin in meat and become more stable. It can be used as a color retention agent in the food processing industry to maintain the good appearance of meat products, prevent the production of Clostridium botulinum, and improve the safety of edible meat products. However, long-term intake of excessive nitrite food can induce canceration of the digestive system. CheKine™ Micro Food Nitrite Assay Kit provides a simple, convenient and rapid method for the detection of nitrite content. The detection principle is that under acidic conditions, nitrite reacts with p-aminobenzenesulfonic acid to form diazo compounds, and then reacts with NMe1-1-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
Extraction Solution I	50 mL	RT
Extraction Solution II	50 mL	RT
Extraction Solution III	50 mL	RT
Activated Carbon	1	RT
Reagent I	10 mL	4°C, protected from light
Reagent II	10 mL	4°C, protected from light
NaNO <sub>2</sub> Standard (1 M)	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, water bath
- Deionized water
- Mortar

## Reagent Preparation

**Extraction Solution:** Ready to use as supplied. Store at room temperature.

**Activated Carbon:** Ready to use as supplied. Store at room temperature.

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

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

**Standard Curve Setting:** 1 M NaNO<sub>2</sub> Standard was diluted to 100 µM with deionized water. Dilute the 100 µM NaNO<sub>2</sub> Standard with deionized water to 50, 25, 12.5, 6.25, 3.125, 1.563 µM as indicated in the table below.

Num.	Volume of Standard	Volume of Deionized Water (µL)	The Concentration of Standard (µM)
Std.1	200 µL of 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 (3.123 µM)	100	1.563

## Sample Preparation

Weigh the sample about 0.2 g fresh weight or 0.05 g dry weight, crush, add 0.5 mL Extraction Solution I , boiling water bath 15 min, cool to room temperature, add 0.5 mL Extraction Solution II , shake well, add 0.5 mL Extraction Solution III , use tweezer to add a small amount of activated carbon (about 1 mg), mix well, rest for 30 min, 8,000 g centrifuge at 25°C for 15 min, take the supernatant to be determined.

**Note:** After adding Extraction Solution II and Extraction Solution III , the sample will be sticky, which is a normal phenomenon.

## 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	Test Well (µL)	Standard Well (µL)	Blank Well (µL)
Sample	70	0	0
Standard	0	70	0
Deionized Water	0	0	70
Reagent I	65	65	65
Reagent II	65	65	65

Mix well and then incubate for 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  $\Delta A_{Test}$  is less than 0.001, increase the sample quantity appropriately. If  $\Delta A_{Test}$  is greater than 1.0, the sample can be appropriately diluted with deionized water, 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  $\Delta A_{\text{Standard}}$  as the x-axis, draw the standard curve. Substitute the  $\Delta A_{\text{Test}}$  into the equation to obtain the y value ( $\mu\text{M}$ ).

### 2. Calculation of $\text{NO}_2^-$ in samples

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

Where:  $V_{\text{Total sample}}$ : Total sample volume, 1.5 mL; 46:  $\text{NO}_2^-$  relative molecular weight, g/mol; W: sample weight, g; n: dilution factor.

## Typical Data

Typical standard curve:

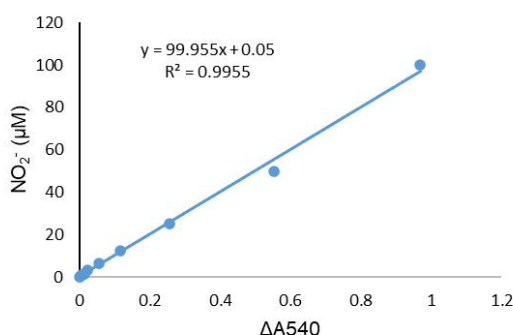


Figure1. Standard curve for  $\text{NO}_2^-$ .

Examples

1. Take 0.2 g pickles and use 96-well plate to calculate  $\Delta A=0.103$ ,  $y=10.345$ . The content calculated according to the sample quality is as follows:

$$\text{NO}_2^- (\mu\text{g/g}) = 0.069 \times 10.345 \div 0.2 \times 1 = 3.569 \mu\text{g/g}.$$

2. Take 0.2 g canned meat and use 96-well plate to calculate  $\Delta A=0.003$ ,  $y=0.75$ . The content calculated according to the sample quality is as follows:

$$\text{NO}_2^- (\mu\text{g/g}) = 0.069 \times 0.75 \div 0.2 \times 1 = 0.259 \mu\text{g/g}.$$

## Recommended Products

Catalog No.	Product Name
KTB3040	CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase (GDH) Assay Kit
KTB3050	CheKine™ Micro Water and Soil Nitrite Content Assay Kit
KTB3070	CheKine™ Micro Urease Activity Assay Kit

## Disclaimer

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