EZMeta™ Food Nitrite Colorimetric Assay Kit

Cat #: D-AKC3051

Size: 96T

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

Product Information

Applicable samples: Food

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. EZMeta™ Food Nitrite Colorimetric 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 NMel-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
$NaNO_2$ 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 $NaNO_2$ Standard was diluted to 100 μM with deionized water. Dilute the 100 μM $NaNO_2$ 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 SolutionⅢ, 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 SolutionⅢ, 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 ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μM).

2. Calculation of NO_2^- in samples

NO_2^- (μg/g)=y×$V_{Total\ sample}$×46÷1,000÷W×n**=0.069×y÷W×n**

Where: $V_{Total\ sample}$: Total sample volume, 1.5 mL; 46: NO_2^- relative molecular weight, g/mol; W: sample weight, g; n: dilution factor.

Typical Data

Typical standard curve:

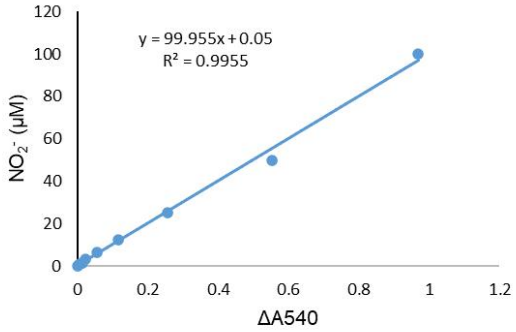

Figure1. Standard curve for NO_2^-.

Examples

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

NO_2^- (μg/g)=0.069×10.345÷0.2×1=3.569 μg/g.

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

NO_2^- (μg/g)=0.069×0.75÷0.2×1=0.259 μg/g.

Disclaimer

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