Nitrite Assay Kit

(Cat/No.:BC022 Size:100T/96S)

1. Assay principle:(Colorimetric method)

Nitrite can react with chromogenic agent to produce pink azo compound, it is able to calculate nitrite content by colorimetry.

1. Composition & preparation (The kit is valid for 6 months)

Reagent 1: Liquid 100ml×1 bottle, can be stored at 2℃-8℃.

Reagent 2: Liquid 50ml×1 bottle, can be stored at 2℃-8℃.

Reagent 3: Powder, when use, add 30ml hot distilled water (≥60℃), dissolve completely. Can be stored at 2℃-8℃ away from light.

Reagent 4: Powder, when use, add 12ml distilled water, dissolve completely. Can be stored at at 2℃-8℃ away from light.

Reagent 5: Solution 12ml×1 bottle, can be stored at room temperature or 2℃-8℃.

Chromogenic agent preparation: V(Reagent 3): V(Reagent 4):V(Reagent 5)=2.5:1:1, store the prepared Chromogenic agent at 2°C–8°C away from light. Discard once it turns dark brown. For repeated use, inspect for crystallization before each application. If crystals form, heat the solution above 60°C to dissolve them, then cool to room temperature before use.

2mmol/ sodium nitrite standard stock solution: 1ml×1 vial, can be stored at 2℃-8℃; (For preparing standard curves)

100μmol/L sodium nitrite standard stock solution: 10ml×1 vial, can be stored at 2℃-8℃.

2. Required EQUIPMENTS & REAGENTS

Spectrophotometer (wavelength: 550 nm) with 1 cm optical path cuvettes, or microplate reader with 96-well plates; 37℃ water bath; desktop low-speed centrifuge; pipettes of various specifications; vortex mixer; centrifuge tubes; electronic balance (milligram level)

3. Operation Procedure

1, Sample Pretreatment

Serum/Plasma and Other Liquid Samples: Use directly without pretreatment.

Cell Culture Medium: Pipette an appropriate amount, centrifuge at 4000 rpm for 5 minutes, and take the supernatant for detection.

Animal Tissue Samples: Accurately weigh the tissue, add 9 volumes of normal saline at a ratio of weight (g) : volume (mL) = 1:9. Perform mechanical homogenization on an ice bath, centrifuge at 4000 rpm for 10 minutes, and take the supernatant for testing (the protein concentration of the supernatant needs to be determined; protein assay kits are available from our company).

Plant Tissue Samples:

Method 1: First, clean the plant tissue with PBS, blot dry with absorbent paper, cut into pieces and put into a mortar, grind into powder with liquid nitrogen. Weigh the plant powder, add 9 volumes of PBS at a ratio of weight (g) : volume (mL) = 1:9, vortex shake (or grind with a grinder) for 1 minute, centrifuge at 4000 rpm for 10 minutes, and take the supernatant for testing.

Method 2: After cleaning and blotting dry, weigh directly, add 9 volumes of PBS at a ratio of weight (g) : volume (mL) = 1:9. Perform mechanical homogenization on an ice bath, centrifuge at 4000 rpm for 10 minutes, and take the supernatant for testing.
Note: Method 2 is recommended for plants with high water content; Method 1 is recommended for plants with low water content or dry samples.

Cell Samples: After collecting cells (the number of cells should not be less than 10^6, the more the better), add 0.3mL normal saline (or PBS) to each sample. Perform ultrasonic disruption on an ice bath (power: 200-300W, work for 5 seconds, interval for 15 seconds, repeat 3-5 times), centrifuge at 4000 rpm for 10 minutes, and take the supernatant for testing (the protein concentration of the supernatant needs to be determined; protein assay kits are available from our company).

4. Operation Table

	Blank tube	Standard tube	Sample tube
Distilled water (ml)	a*		
100μmol/L sodium nitrite standard (ml)		a*	
Sample（ml）			a*
Reagent 1 (ml)	0.8	0.8	0.8
Reagent 2 (ml)	0.4	0.4	0.4
Mix thoroughly with a vortex mixer, incubate at room temperature for 10 minutes, centrifuge at 3500 rpm at room temperature for 10 minutes, take 0.8mL of the clear supernatant for chromogenic reaction			
Supernatant (ml)	0.8	0.8	0.8
Chromogenic agent (ml)	0.4	0.4	0.4
Mix sufficiently, wait for 15 minutes, transfer in cuvettes of 0.5cm light path, measure OD valu es of all tubes at 550nm (adjust zero by distilled water)			

Note: a* refers to the volume of sample, standard solution, and double distilled water (the three are equal). Recommended sampling volume:

Serum/plasma: 0.1 ~ 0.4mL

Tissue/cell homogenate: 0.5 ~ 0.8mL

Cell culture medium: 0.5 ~ 1mL

5. Calculation

Formula

For Liquid samples:

$$\text{Nitrite content}(\mu\text{mol/L}) = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times N$$

For Animal Tissue/Cell Samples:

$$\text{Nitrite content}(mol/gprot) = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div C_{pr}$$

For Plant Tissue Samples:

$$\text{Nitrite content}(\mu\text{mol}/g) = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div \frac{\text{W}}{\text{V}_{\text{Total sample}}}$$

For Cell Samples:

$$\text{Nitrite content}(\mu\text{mol}/10^{4} cells) = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \div \frac{\text{Total Cell Count}}{\text{V}_{\text{Total sample}}}$$

Parameter Definitions

In the above formula:

$C_{standard}$ is the standard solution concentration, 100 μmol/L;

N is the dilution factor prior to sample testing;

C_{pr} is the protein concentration in the tissue or cell homogenate, gprot/L (prot denotes protein);

W is the sample mass, g;

$V_{Total\ sample}$ is the total volume of solution during sample pretreatment (approximately equal to the total volume of extraction solution added), L;

Total cell count: the total number of collected cells, in 10^4 cells.

Note: Animal tissues may also refer to the plant calculation formula; either method may be selected for cell calculations.

6. Announcements

1) Strictly follow the operating procedures. It is recommended to use disposable plastic test tubes or centrifuge tubes for operation.
2) The supernatant after centrifugation must be clear; re-centrifuge if turbid.
3) High lipid content in samples will affect the determination results (add an equal volume of chloroform to the sample before centrifugation, vortex for 30-60 seconds, then centrifuge).
4) Serum and tissue blocks can be stored frozen below 0℃ for more than half a month. The lower the temperature, the longer the storage period. Samples can be stored at 2 ~ 8℃ for 3 days.
5) It is best to prepare reagents one day before testing to ensure complete dissolution. Before centrifugation, mix thoroughly with a vortex mixer and let stand for at least 10 minutes.
6) For the final colorimetry, either a spectrophotometer (equipped with 1mL cuvettes) or a microplate reader (pipette 200μL for reading) can be used.
7) This kit is for research use only.

7. Standard Curve Preparation(Optional)

8. Dilute 2 mmol/L sodium nitrite standard stock solution with distilled water to concentrations of 0, 10, 25, 50, 75, 100 and 200 μmol/L. Operate according to the standard tube sampling in the operation table, measure the OD value at each concentration. Subtract the OD value of 0-concentration tube from that of each concentration tube (including

0-concentration tube), fit a standard curve with the corresponding standard concentrations, and calculate with the obtained formula (calculation results replace the $\frac{A_{\text{sample}}-A_{\text{blank}}}{A_{\text{standard}}-A_{\text{blank}}} \times C_{\text{standard}}$).