Nitrite Assay Kit

ARG83399 Nitrite Assay Kit can be used to measure Nitrite in serum, plasma, urine, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83399

Package: 96 wells

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INTRODUCTION

Nitrite can be reduced to nitric oxide or ammonia by many species of bacteria. Under hypoxic conditions, nitrite may release nitric oxide, which causes potent vasodilation. Several mechanisms for nitrite conversion to NO have been described, including enzymatic reduction by xanthine oxidoreductase, nitrite reductase, and NO synthase (NOS), as well as nonenzymatic acidic disproportionation reactions.

PRINCIPLE OF THE ASSAY

The ARG83399 Nitrite Assay Kit determined Nitrite by nitrite is reduced to Nitrogen Oxide using Griess Reagent I. Then, Nitrogen Oxide reacts with Griess Reagent II forming a stable product. The increase in absorbance at 540 nm is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Reaction Dye Diluent	3 ml	4°C
Reaction Dye A	1 vial (lyophilized)	4°C (protect from light)
Reaction Dye B	1 vial (lyophilized)	4°C (protect from light)
Assay Buffer A	20 ml	4°C
Assay Buffer B	20 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all component at 4°C. Reaction Dye A and B should protect from light.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For tissue- Weigh out 0.1 g tissue, homogenize with 0.8 ml distilled water, transfer all samples into centrifuge tube, add 0.1 ml Assay Buffer A, mix; then add 0.1 ml Assay Buffer B, mix; centrifuged at 10,000 rpm 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection

For liquid- Add 0.8 ml samples into centrifuge tube, add 0.1 ml Assay Buffer A, mix; then add 0.1 ml Assay Buffer B, mix; centrifuged at 10,000 rpm 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to yield stock, then add **10 µl** standard into **990 µl distilled water** to yield standard stock, then add **1 µl** stock into **999 µl distilled water** to yield **1 nmol/ml** standard. Perform 2-fold serial dilution of the top standards using distilled water to make the standard curve. The concentration of standard curve could be **1 nmol/ml, 0.5 nmol/ml, 0.25 nmol/ml, 0.125 nmol/ml, 0.0625 nmol/ml, 0.0312 nmol/ml, 0.0156 nmol/ml.**
- **Reaction Dye A:** Reconstitute the Substrate with **1 ml** of Reaction Dye Diluent. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Reaction Dye B:** Reconstitute the Substrate with **1 ml** of Reaction Dye Diluent. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Control**: If Assay Buffer A and Assay Buffer B were used for sample deproteinization, Control should be prepared parallelly by mixing **800 ul** distilled water with **100 µl** Assay Buffer A and **100 µl** Assay Buffer B. Centrifuge at 10,000 rpm for 10 minutes, take the supernatant as the control.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **Distilled water** before assay and assay again.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **180 µl Samples** into Sample wells.
2. Control wells: Add **180 µl Control** into Control wells
3. Standard wells: Add **180 µl Standard** into Standard wells.
4. Blank wells: Add **180 µl Distilled water** into Blank wells.
5. Add **10 µl** per **Reagent Dye A** into each wells.
6. Mix well. Incubate at **RT** for **3 min**.
7. Add **10 µl** per **Reagent Dye B** into each wells.
8. Mix well. Incubate at **RT** for **15 min**. Read the OD at **540 nm**.

Summary of Nitrite Assay Kit Procedure

Reagent	Sample	Control	Standard	Blank
Sample	180 µl	-	-	-
Control	-	180 µl	-	
Standard	-	-	180 µl	-
Distilled water	-	-	-	180 µl
Reagent Dye A	10 µl	10 µl	10 µl	10 µl
Mix well. Incubate all Sample tubes at **RT** oven for **3 min**.				
Reagent Dye B	10 µl	10 µl	10 µl	10 µl
Mix well. Incubate at **RT** oven for **15 min**.				
Read the OD with a microplate reader at **540 nm**.				

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 1 nmol/ml;

V_{Assay}: the volume of distilled water and assay buffer, 1 ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 180 µl = 0.18 ml;

$V_{standard}$: the volume of standard sample, 180 µl = 0.18 ml;

n: dilution factor; =2

B. Formula:

a). According to the weight

Nitrite (nmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{total})]$

$= (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$

b). According to the volume

Nitrite (nmol/g) =

$N \times [(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

$=2 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank})$

3. Detection range:

The detection range is from 0.01 nmol/ml - 1 nmol/ml.

4. If the samples have been diluted, the calculated concentration must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serially diluted standards are necessary for this kit.