Nitrite Microplate Assay Kit

User Manual

Catalog # CAK1194

(Version 1.2A)

Detection and Quantification ofNitriteContentin Serum, Plasma, Urine, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Nitrogen-based ions, nitrite and nitrate, are found in almost every living organism. Furthermore, endogenous Nitrite levels are found in mammals and also can be obtained from dietary sources. In humans, nitrite is further metabolized to Nitric Oxide and other reactive nitrogen species (nitrogen oxides). The Nitrate-Nitrite-NO biochemical pathway is well known for its participation in cell signaling, hypoxia-dependent response and regulation of blood flow. Recent studies suggest the reduction of Nitrite to Nitrogen Oxygen in the mitochondria. Specifically, myoglobin and xanthine oxidoreductase could generate NO under hypoxic conditions leading to mitochondrial respiration.

Nitrite Microplate Assay Kit utilizes the Griess Reagent, a classic protocol for the estimation of nitrite. In the assay, nitrite is reduced to Nitrogen Oxide using Griess Reagent I. Then, Nitrogen Oxide reacts with Griess Reagent II forming a stable product that can be detected by its absorbance at OD 540 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	30 ml x 1	4 °C
Dye ReagentDiluent	3 ml x 1	4 °C
Dye Reagent A	Powderx 1	4 °C
Dye Reagent B	Powderx 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Dye Reagent A: add 2 mlDye ReagentDiluentto dissolve before use.

Dye Reagent B: add 1 mlDye ReagentDiluentto dissolve before use.

Standard: add 1 mldistilled water to dissolve before use, add 10µlstandard into 990µldistilled water, mix; then add 1µldiluted standard into 999µldistilled water, mix; the concentration will be 1nmol/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1.For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5mldistilled water, transfer all samples into centrifuge tube, add 0.25 ml Assay Buffer I, mix; then add 0.25 ml Assay Buffer II, mix;centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2.For liquid samples

Add 0.5ml samples into centrifuge tube, add 0.25 ml Assay Buffer I, mix; then add 0.25 ml Assay Buffer II, mix; centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	170 µl	--	--
Standard	--	170 µl	
Distilled water	--	--	170 µl
Dye Reagent A	20 µl	20 µl	20 µl
Mix well, incubate at room temperature for 3 minutes.			
Dye Reagent B	10 µl	10 µl	10 µl
Mix, incubate at room temperature for15 minutes, record absorbance measured at 540 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

Nitrite (nmol/g) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$

$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

2.According to the volume of sample

Nitrite (nmol/ml) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$

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

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

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.17 ml;

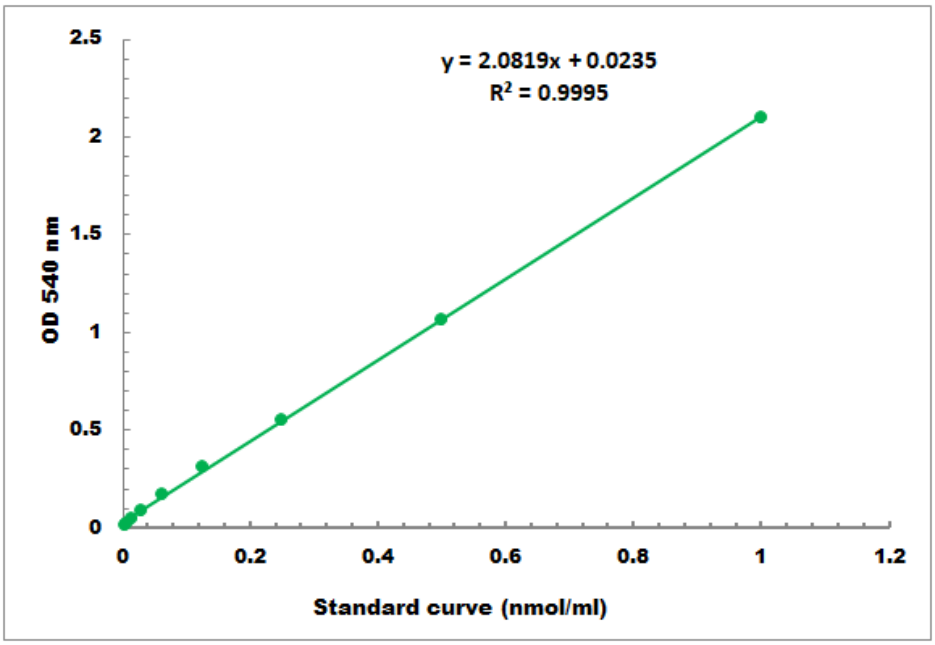
$V_{Standard}$: the volume of standard, 0.17 ml;

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

n: dilution factor, n=2.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 nmol/ml-1nmol/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES