Nitrite

ColorimetricMicroplate Assay Kit

User Manual

Catalog # CAK1194

(Version 1.3B)

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.....2
II. KIT COMPONENTS.....3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....4
IV. REAGENT PREPARATION.....5
V. SAMPLE PREPARATION.....6
VI. ASSAY PROCEDURE.....7
VII. CALCULATION.....8
VIII.TYPICAL DATA.....9

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 mitochondrial. Specifically, myoglobin and xanthine oxidoreductase could generate NO under hypoxic conditions leading to mitochondrial respiration.

Nitrite ColorimetricMicroplate 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	20 ml x 1	4 °C
Assay Buffer II	20 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
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Dye Reagent A: Briefly centrifuge prior to opening. Add 1 mlDye ReagentDiluentto dissolve before use.Store at -20 °C for 1 month.

Dye Reagent B: Briefly centrifuge prior to opening. Add 1 mlDye ReagentDiluentto dissolve before use.Store at -20 °C for 1 month.

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. Store at -20 °C for 1 month. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/0.0312/0.0156 nmol/ml.

V. SAMPLE PREPARATION

1.For tissue samples

Weigh out 0.1 g tissue, homogenize with 800 µl distilled water, transfer it into the centrifuge tube;then add 100 µlAssay Buffer I mix, and 100 µl Assay Buffer II mixagain, centrifuged at 10,000 rpm for 10minutes, take the supernatant into a new centrifuge tube for detection.

2.For liquid samples

If the sample does not contain any protein or less protein, it can be assayed directly.

If the sample contains more protein,the samples should be cleared by mixing 800 µlsample with 100 µlAssay Buffer I and 100 µl Assay Buffer II.Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a cleantubefor detection (dilution factor n = 1.25).

Note: If assay bufferI and II were used for sample deproteinization, **Control** should be prepared parallelly by mixing 800 µldistilled water with100 µlAssay Buffer Iand 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm,take the supernatant as the control.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Control	Standard	Blank
Sample	180 µl	--	--	--
Control	--	180 µl	--	--
Standard	--	--	180 µl	
Distilled water	--	--	--	180 µl
Dye Reagent A	10 µl	10 µl	10 µl	10 µl
Mix well, incubate at room temperature for 3 minutes.				
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 15 minutes, record absorbance measured at 540 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Control})-\text{Intercept}}{\text{Slope}}\times n(nmol/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1. According to the weight of sample

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

2.2.According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

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

W: the weight of sample, g

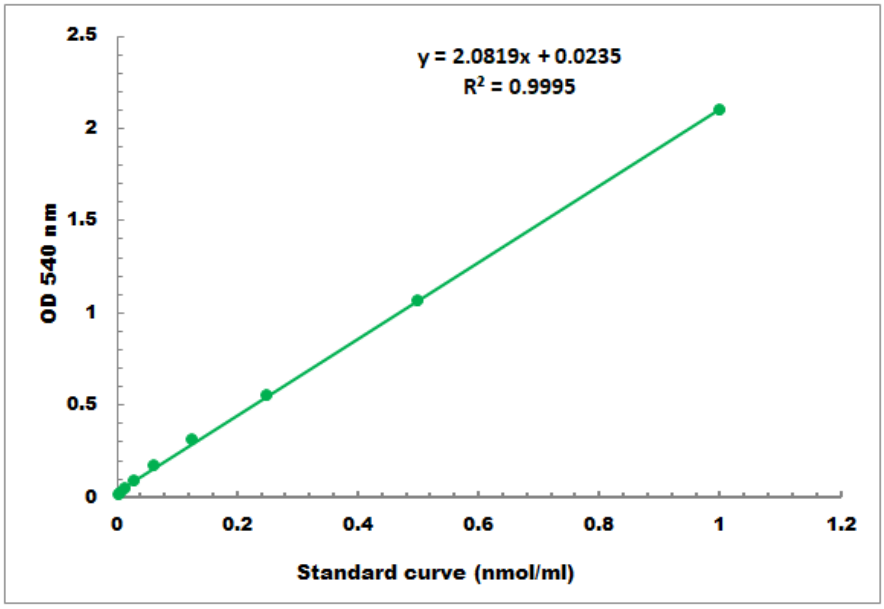
V_{Sample}: the volume of sample, ml

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

V_{Assay}: the volume of distilled water, Assay Buffer I and Assay Buffer II, mL

VIII. 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