Nitrite Reductase

Microplate Assay Kit

User Manual

Catalog # FTA0198

(Version 1.2A)

Detection and Quantification of Nitrite Reductase (NiR) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Nitrite reductase refers to any of several classes of enzymes that catalyze the reduction of nitrite. There are two classes of NIR's. A multi haem enzyme reduces NO_2^- to a variety of products. Copper containing enzymes carry out a single electron transfer to produce nitric oxide.

Nitrite Reductase Microplate Assay Kit is a sensitive assay for determining Nitrite Reductase activity in various samples. Nitrite reductase can reduce NO_2^- to NO. NO_2^- can react with dye reagent, and can be measured at a colorimetric readout at 540 nm. The reduction of NO_2^- is proportional to the nitrite reductase activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	6 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Substrate: add 2 ml distilled water to dissolve before use.

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 20 µl into 980 µl distilled water. The concentration will be 2 mmol/L.

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. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 4000g 4 °C for 5 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 4000g 4 °C for 5 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl	60 µl
Substrate	20 µl	20 µl	--	--
Sample	20 µl	--	--	--
Standard	--	--	20 µl	--
Distilled water	--	20 µl	20 µl	40 µl
Mix, put it in the oven, 37 °C for 30 minutes.				
Stop Solution	50 µl	50 µl	50 µl	50 µl
Mix, centrifuged at 10,000g 4 °C for 10 minutes, add the supernatant into the microplate.				
Supernatant	100 µl	100 µl	100 µl	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, record absorbance measured at 540 nm.				

Note:

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

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of NiR activity is defined as the enzyme reduce 1 μmol of NO_2^- per hour.

1. According to the protein concentration of sample

$$NiR\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 4 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$NiR\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W/ V_{Assay}) / T$$

$$= 4 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$NAG\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N/ V_{Assay}) / T$$

$$= 4 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of standard, 2 mmol/L = 2 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.02 ml;

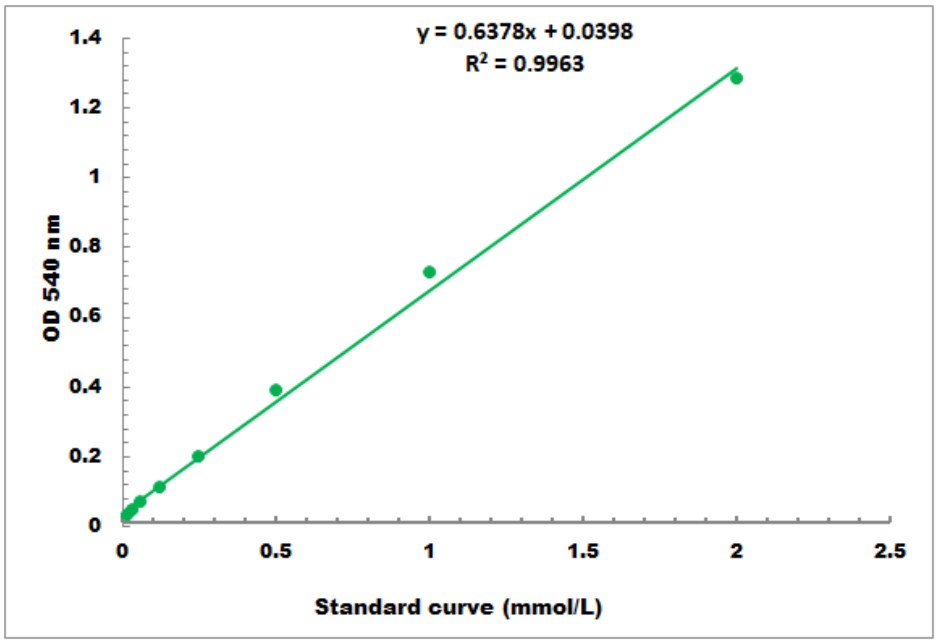
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes = 0.5 hour.

VII. TYPICAL DATA

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



Detection Range: 0.02 mmol/L - 2 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES