Nitrite reductase Assay Kit

ARG83413 Nitrite reductase Assay Kit can be used to measure Nitrite reductase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83413

Package: 96 wells

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INTRODUCTION

Nitrite reductase (NIR) has been determined to be the major NO producer in mammalian cells under hypoxic conditions, such as those that commonly occur in hypoxic vasodilation of cardiovascular systems, muscle tissue during extensive exercise, and ischemic tissues. Unlike NOS, NIR produces NO in an O2-independent manner, suggesting the potential contribution of NO in mediating hypoxic signalling-dependent cellular responses. Haem-binding proteins, such as haemoglobin and myoglobin, and cytochrome c, cytochrome bc1, and cytochrome c oxidase of the mitochondrial electron transport chain, have been reported to generate NO via NIR-dependent catalysis.

PRINCIPLE OF THE ASSAY

Nitrite reductase Assay Kit determined Nitrite reductase activit y in various samples. Nitrite reductase can reduce $NO2^-$ to NO. $NO2^-$ can react with dye reagent. The reduction of $NO2^-$ is proportional to the nitrite reductase activity can be measured at a colorimetric readout at 540 nm

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Reaction Buffer	6 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Reagent Dye Diluent	10 ml	4°C
Stop Solution	5 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- 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.
- 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 cell and bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 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

For tissue - 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.

REAGENT PREPARATION

- Standard: Reconstitute the Standard with 1 ml of distilled water. Allow the Standard keep on bench for few minutes make sure the Standard is dissolved completely. Then add 20 µl Standard into 980 µl distilled water. The concentration will be 2 µmol/ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- Substrate: Reconstitute the Substrate with 2 ml of distilled water. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- Dye Reaction: Add 10 ml Reagent Dye Diluent before use. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **60 µl Reaction Buffer** into each tubes.
2. Add **20 µl Substrate** into Sample and Control tubes.
3. Add **20 µl Sample** into Sample tubes.
4. Add **20 µl Standard** into Standard tubes.
5. Add **20 µl Distilled water** into Control, Standard tubes.
6. Add **40 µl Distilled water** into Blank tubes.
7. Mix well. Incubate at **37°C** for **30 min**.
8. Add **50 µl Stop Solution** into all tubes.
9. Brief spin the tubes at 10,000g 4 °C for 10 minutes and transfer 100 µl Supernatant the reagents into the 96-well microplate.
10. Add **100 µl Reagent Dye** into all wells.
11. Mix well. Read the OD at **540 nm**.

Summary of Nitrite reductase Assay Procedure

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 well. Incubate at 37°C for 30 min				
Stop Solution	50 µl	50 µl	50 µl	50 µl
Brief spin the tubes at 10,000g 4 °C for 10 minutes. Transfer Supernatant into the 96-well microplate.				
Supernatant	100 µl	100 µl	100 µl	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix well. Read the OD at 540 nm.				

CALCULATION OF RESULTS

1. Unit Definition: One unit Nitrite reductase activity is defined as the generates 1 μmol of $NO2^-$ per hour in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the concentration of Standard, 2 μmol/ml;

V_{Sample}: the volume of reaction sample, 20 μl = 0.02 ml;

$V_{Standard}$: the volume of reaction Standard, 20 μl = 0.02 ml;

V_{assay}: the volume of Assay Buffer, 1000 μl = 1 ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

T: the reaction time, 30 minutes.

B. Formula:

a). According to the protein concentration of sample

Nitrite reductase activity (U/mg) =

$(OD_{Control} - OD_{Sample}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

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

b). According to the weight of sample

Nitrite reductase activity (U/mg) =

$(OD_{Control} - OD_{Sample}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

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

c). According to the cell or bacteria

Nitrite reductase activity ($U/10^4$) =

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

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