Nitrate Reductase Assay Kit

Nitrate Reductase Assay Kit is a detection kit for the quantification of Nitrate Reductase Activity in tissue extracts and cell lysate.

Catalog number: ARG82016

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Nitrate reductases are molybdoenzymes that reduce nitrate (NO_3^-) to nitrite (NO_2^-). This reaction is critical for the production of protein in most crop plants, as nitrate is the predominant source of nitrogen in fertilized soils.

Eukaryotic nitrate reductases are part of the sulfite oxidase family of molybdoenzymes. They transfer electrons from NADH or NADPH to nitrate.

Prokaryotic nitrate reductases belong to the DMSO reductase family of molybdoenzymes and have been classified into three groups, assimilatory nitrate reductases (Nas), respiratory nitrate reductase (Nar), and periplasmic nitrate reductases (Nap). The active site of these enzymes is a Mo ion that is bound to the four thiolate functions of two pterin molecules. The coordination sphere of the Mo is completed by one amino-acid side chain and oxygen and/or sulfur ligands. The exact environment of the Mo ion in certain of these enzymes (oxygen versus sulfur as a sixth molybdenum ligand) is still debated. The Mo is covalently attached to the protein by a cysteine ligand in Nap, and an aspartate in Nar. [Provide by Wikipedia: Nitrate reductase]

PRINCIPLE OF THE ASSAY

This Nitrate Reductase Assay Kit is a simple colorimetric assay that measures the amount of Nitrate Reductase present in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. The assay is initiated with the enzymatic hydrolysis of the nitrate by Nitrate Reductase. The enzyme catalysed reaction products azo-compound can be measured at a colorimetric readout at 540 nm. The concentration of Nitrate Reductase in the samples is then determined by comparing the O.D. 540 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Substrate I	6 mL	4°C
Substrate II (lyophilized)	1 vial	-20°C
Dye Reagent I	10 mL	4°C
Dye Reagent II	10 mL	4°C
Standards (8 µmol/mL)	1 mL	4°C
Plate sealer	3 ea	RT
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 510 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Liquid samples: To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly.

REAGENT PREPARATION

- **Substrate II:** Add 2 mL of distilled water to dissolve before use.
- **Standards:** Use the 8 μmol/mL Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (μmol/mL)	Volume of distilled water (μL)	Volume of 8 μmol/mL Standards (μL)
S1	8	0	500
S2	4	250	250 of S1
S3	2	250	250 of S2
S4	1	250	250 of S3
S5	0.5	250	250 of S4
S6	0.25	250	250 of S5

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **20 µL** of **samples, Blank (distilled water), Control** or serial **diluted Standards** into 96-well microplate.
2. Add **80 µL** of Distilled water to the Control well.
3. Add **60 µL** of **Substrate I** into each well (without Control well).
4. Add **20 µL** of **Substrate II** into each well (without Control well).
5. Mix well and incubate for **30 minutes** at **37°C** in the oven.
6. Add **50 µL** of **Dye Reagent I** into each well.
7. Add **50 µL** of **Dye Reagent II** into each well.
8. Mix well, and incubate for **20 minutes** at **room temperature.**
9. Read the plate with a microplate reader at **540 nm**.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One unit of NR activity is defined as the enzyme generates 1 µmol of NO^{2-} per hour.

5. According to the protein concentration of sample:

NR (U/mg)

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

$= [16 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

6. According to the weight of sample:

NR (U/g)

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

$= [16 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / W$

Note:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 8 μmol/mL;

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

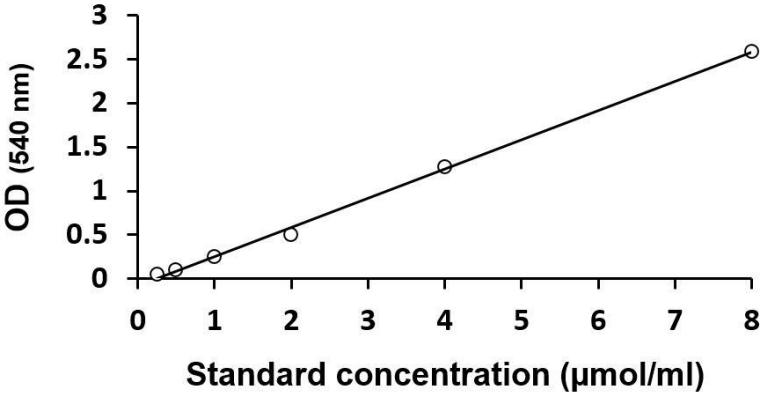
V_{Sample}: the volume of sample, 0.02 mL;

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

T: the reaction time, 0.5 hour.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Nitrate Reductase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 µmol/mL