Nitrate Reductase

Microplate Assay Kit

User Manual

Catalog # FTA0014

(Version 1.2E)

Detection and Quantification of Nitrate Reductase (NR) Activity in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Nitrate Reductase (NR), also known as respiratory nitrate reductase. 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. Nitrate reductase activity can be used as a biochemical tool for predicting grain yield and grain protein production. Nitrate reductase promotes amino acid production in tea leaves. It is reported that tea plants sprayed with various micronutrients (like Zn, Mn and B) along with Mo enhanced the amino acid content of tea shoots and also the crop yield.

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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate I	6 ml x 1	4 °C
Substrate II	Powder x 1	-20 °C
Dye Reagent I	10 ml x 1	4 °C
Dye Reagent II	10 ml x 1	4 °C
Standard (8 µmol/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate II: add 2 ml distilled water before use.

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 tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	20 µl	--	--
Standard Solution	--	--	20 µl	--
Distilled water	--	80 µl	--	20 µl
Substrate I	60 µl	--	60 µl	60 µl
Substrate II	20 µl	--	20 µl	20 µl
Mix, put it in the oven, 37 °C for 30 minutes.				
Dye Reagent I	50 µl	50 µl	50 µl	50 µl
Dye Reagent II	50 µl	50 µl	50 µl	50 µl
Mix, wait for 20 minutes, 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 NR activity is defined as the enzyme generates 1 μmol of NO^{2-} per hour.

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

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

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

W: the weight of sample, g;

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

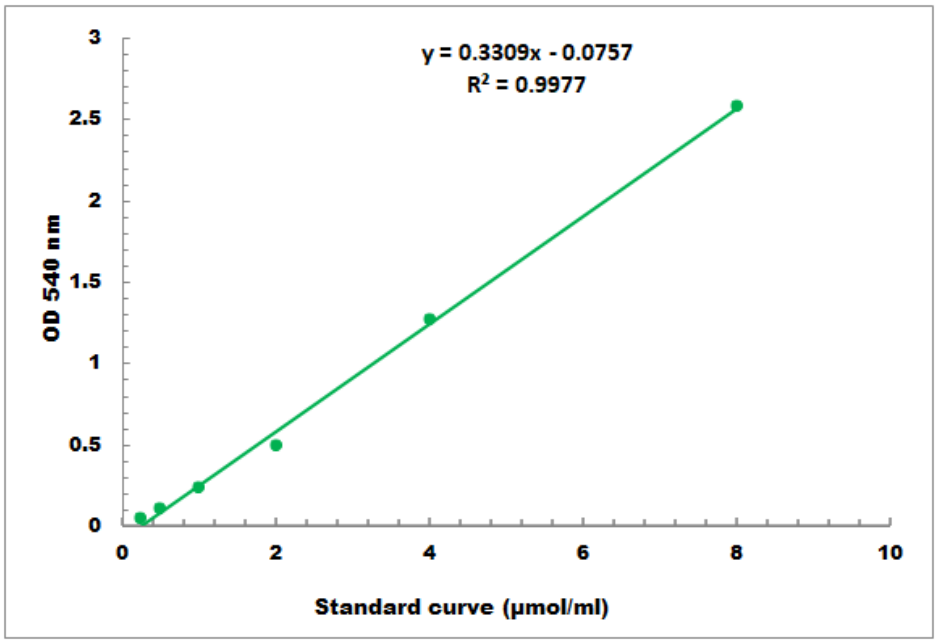
V_{Sample}: the volume of sample, 0.02 ml;

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

T: the reaction time, 0.5 hour.

VII. TYPICAL DATA

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



Detection Range: 0.1 µmol/ml - 8 µmol/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES