



Nitrate Reductase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1014

(Version 1.5E)

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

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

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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 product 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 (8mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
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. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 8/4/2/1/0.5/0.25/0.125 mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 2 ml distilled water before use. Keep in dark for immediate use or store at -20 °C for 2-4 weeks.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank
Sample	20µl	--	--	--
Assay Buffer	--	20 µl	--	--
Standard	--	--	20µl	--
Distilled water	--	--	--	20µl
Substrate I	60 µl	60 µl	60 µl	60 µl
Substrate II	20 µl	20 µl	20 µl	20 µl
Mix, put it in the oven, 37°C for 30 minutes.				
Dye Reagent I	50µl	50µl	50µl	50µl
Dye Reagent II	50µl	50µl	50µl	50µl
Mix, wait for 20 minutes, record absorbance measured at 540 nm.				

Note:

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

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One unit of NR activity is defined as the enzyme generates 1 μmol of NO^{2-} per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

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

2.1 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.2 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

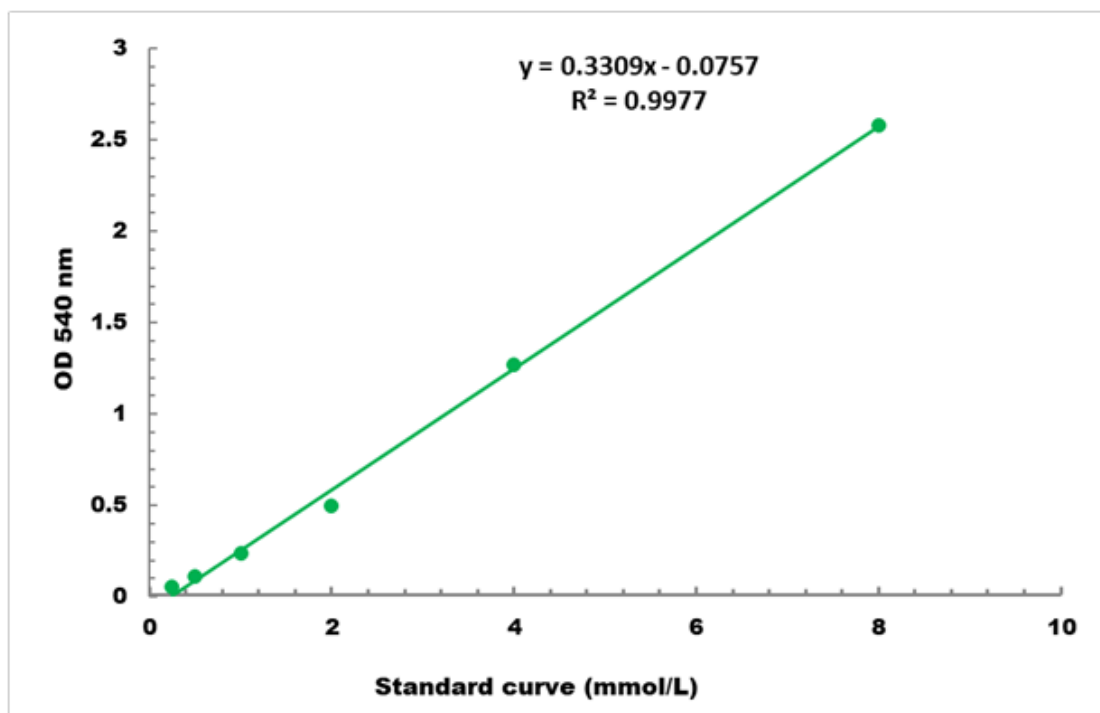
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 8mmol/L