Nitrate Reductase Assay Kit

Catalog No.: abx096013

Size: 100 tests

Detection Range: 1.3 U/L – 250 U/L

Sensitivity: 1.3 U/L

Storage: Store Reagent 1, Reagent 2, and the Standard Solution at -20°C, and all other components at 4°C. Reagent 4 must be kept in the dark.

Application: For detection and quantification of Nitrate Reductase activity in plant tissue, cell culture and bacterial cell samples.

Introduction

The Nitrate Reductases (NR) are a family of molybdenum-containing enzymes used by plants and bacteria to reduce nitrate (NO_3^-) to nitrite (NO_2^-). This is the first step of the nitrate assimilation pathway, which converts organic nitrates present in the soil into a useable source of nitrogen for plant growth.
As Nitrate Reductase is key to the incorporation of nitrogen-containing compounds into the plant, NR activity can be used as a general predictor of crop and grain yield.

Abbexa's Nitrate Reductase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Nitrate Reductase activity. Under acidic conditions, nitrite produced by the action of NR will react with p-aminobenzenesulfonic acid and α-naphthylamine to form red azo compounds, with a peak absorbance at 540 nm. The intensity of the color will be proportional to the activity of the Nitrate Reductase enzyme, which can then be calculated.

Kit components

1. Reagent 1: 10 ml
2. Reagent 2: 5 ml
3. Reagent 3: 6 ml
4. Reagent 4: 6 ml
5. Standard Solution: 1 ml
6. Sample Buffer: 50 ml
7. Extraction Solution: 60 ml
8. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Double-distilled water
3. Absorbent filter paper
4. Pipette and pipette tips
5. Centrifuge tubes
6. Sonicating water bath
7. Centrifuge
8. Microplate shaker

Protocol

A. Preparation of samples and reagents

1. Reagents

- **0.1 µmol/ml Diluted Standard:** Dilute the Standard Solution 100-fold with Double-distilled water (i.e. to the 1 ml stock Standard Solution, add 99 ml Double-distilled water).
- **Working Sample Buffer:** Dilute the Sample Buffer 10-fold with Double-distilled water (i.e. to the 50 ml stock Sample Buffer, add 450 ml Double-distilled water).

2. Samples

Isolate the test samples soon after collecting and analyze immediately.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Cell cultures and bacterial cell samples:** Collect the cells into a centrifuge tube. Centrifuge at 1000 × g for 10 minutes and discard the supernatant. Add Extraction Solution to the remaining precipitate in a ratio of 1 ml Extraction Solution per 5 × 10^6 cells, then sonicate in an ice water bath (for most samples, sonicate at 20% power for 3 seconds, then wait 10 seconds; repeat 30 times). Centrifuge at 8000 × g for 10 minutes at 4 °C. Take the supernatant into a new centrifuge tube, keep on ice, and analyze immediately.
- **Plant Tissue:** Weigh the tissue sample. For each 1 g of tissue, add 10 ml Extraction Solution (it is recommended to use 0.1 g of tissue, adding 1 ml of Extraction Solution). Homogenize manually on ice, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 8000 x g for 10 minutes at 4°C. Take the supernatant into a new centrifuge tube, keep on ice, and analyze immediately.

  If the concentration of NR is predicted to be low (e.g. where $OD_{SAMPLE} \leq OD_{CONTROL}$), before homogenization immerse the fresh tissue for 2 hours in a beaker of Working Sample Buffer. Dry with absorbent filter paper, then perform the protocol above.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

B. Assay Procedure

1. On the 96-well microplate, mark the positions of the sample, control, standard, and blank wells. Each sample tested will require one sample well and one control well. *It is recommended to run these wells in duplicate*.
2. Add 20 µl of sample to the sample and control wells.
3. Add 20 µl Diluted Standard to the standard well.
4. Add 95 µl double-distilled water to the blank well and add 75 µl double-distilled water to the control wells.
5. Add 75 µl Reagent 1 to the sample and standard wells.
6. Add 25 µl Reagent 2 to all wells.
7. Shake the plate gently to mix the contents of the wells fully. For mammalian samples, incubate at 37°C for 30 minutes in the dark. For all other samples, incubate at 25°C for 30 minutes in the dark.
8. Add 50 µl Reagent 3 to all wells.
9. Add 50 µl Reagent 4 to all wells.
10. Mix fully, then incubate at 25°C for 30 minutes in the dark. Measure the absorbance of the samples at 540 nm.

Note:

- Nitrate Reductase degrades in strong light and elevated temperatures. Ensure the plate wells are completely shielded from light during incubation. Perform the assay immediately following sample preparation.

C. Calculation of Results

1. Nitrate Reductase activity according to fresh sample weight

One unit of Nitrate Reductase activity is defined as the amount of Nitrate Reductase in 1 g of sample that can catalyze the production of 1 µmol NO_2^- per hour:

$$\mathrm{NR\ (U = \mu mol/g/h)} = \frac{\mathrm{OD_{Sample} - OD_{Control}}}{\mathrm{OD_{Standard} - OD_{Blank}}} \times \frac{\mathrm{C_{Standard} \times V_{Sample} \times V_{Extract}}}{\mathrm{W \times V_{Sample}}} \times \frac{1}{\mathrm{T}}$$

$$= \frac{0.2}{\mathrm{W}} \times \frac{\mathrm{OD_{Sample} - OD_{Control}}}{\mathrm{OD_{Standard} - OD_{Blank}}}$$

2. Nitrate Reductase activity according to protein concentration in the sample

One unit of Nitrate Reductase activity is defined as the amount of Nitrate Reductase in 1 mg of tissue protein that can catalyze the production of 1 µmol NO_2^- per hour:

$$\mathrm{NR\ (U = \mu mol/mg/h)} = \frac{\mathrm{OD_{Sample} - OD_{Control}}}{\mathrm{OD_{Standard} - OD_{Blank}}} \times \frac{\mathrm{C_{Standard} \times V_{Sample}}}{\mathrm{C_{Protein} \times V_{Sample}}} \times \frac{1}{\mathrm{T}}$$

$$= \frac{0.2}{\mathrm{C_{Protein}}} \times \frac{\mathrm{OD_{Sample} - OD_{Control}}}{\mathrm{OD_{Standard} - OD_{Blank}}}$$

where:

OD_{Sample}	OD value of the sample well
$OD_{Control}$	OD value of the corresponding control well
$OD_{Standard}$	OD value of the standard well
OD_{Blank}	OD value of the blank well
$C_{Standard}$	concentration of the standard (0.1 µmol/ml)
$C_{Protein}$	concentration of the protein in the sample (µmol/ml)
V_{Sample}	volume of sample (0.02 ml)
$V_{Extract}$	volume of Extraction Solution (1 ml)
T	final incubation time (0.5 hours)