# Nitrate Reductase (NR)Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Catalog Number:** BC0085

**Size:**100T/96S

**Components:**

**Inducer reserve fluid:** Liquid 100 mL×1. Storage at 2-8℃. It should be prepared before using with 10 times dilution. Prepare the reagent when it will be used.

**Extraction reagent:** Liquid 120 mL×1 . Storage at 2-8℃.

**Reagent I:** Liquid 15 mL×1. Storage at -20℃.

**Reagent II:** Powder ×1. Storage at -20℃. Add 2 mL of Extraction reagent before using, mix thoroughly. For long term preservation, separate into small tubules and storage at -20℃, avoid repeated freezing and thawing. It can be stored for two weeks at -20℃.

**Product Description**

NR (EC 1.7.1.3) is a key enzyme in the transformation of plant nitrate nitrogen into ammonia nitrogen as well as an induction enzyme, widely exists in plants and has an impact on crop yield and quality.

NR catalyzed nitrate reduction to nitrite, with $NO_3^-$ + NADH + $H^+$ → $NO_2^-$ + $NAD^+$ + $H_2O$. NADH has a characteristic absorption peak at 340 nm. The change of absorbance at 340 nm can indicate the enzyme activity.

**Reagents and Equipment Required but Not Provided.**

Spectrophotometer/ microplate reader, table centrifuge, water-bath, micro quartz cuvette/96 well UV flat-bottom plate, mortar/ homogenate, ice and distilled water.

**Procedure**

**I. Sample Preparation**

1. Put proper inducers in a beaker, wash fresh specimens and then drain with filter paper. Put the specimens in the inducer applied liquid (covered), protected from light, immerse 2 hours. Take out the samples and drain with filter paper. Frozen at -20℃ for 30 minutes, then take out the sample, drain with filter paper. (Conduct induction treatment as needed)
2. Put 0.1 g of induced sample into 1 mL of Extraction reagent and fully grinding on ice. Centrifuge at 4000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

**II. Determination procedures**

1. Preheat spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm, set spectrophotometer counter to zero with distilled water.
2. Sample determination (adding the following reagents to the EP tube):

| Reagent (μL) | Test Tube ($A_T$) | Blank Tube ($A_B$) |
|---|---|---|
| Sample | 12 | - |
| Extraction reagent | 68 | 80 |
| Reagent I | 108 | 108 |
| Reagent II | 12 | 12 |

Mix thoroughly, detect the initial value at 340 nm and noted as $A_{T1}$, $A_{B1}$. Then react at 25℃ (other species) or 37℃(mammal) for 30 minutes and detect the absorbance at 340 nm and noted as $A_{T2}$/$A_{B2}$. Calculate the change for the samples and blank control: $\Delta A_T=A_{T1}-A_{T2}$, $\Delta A_B=A_{B1}-A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$. Blank tubes only need to be tested 1-2 times.

**III. Calculation**

a. micro quartz cuvette

1. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 μmol of NADH in the reaction system per hour every gram sample.

NR activity (U/g weight) =[ΔA×Vt÷(ε×d) ×$10^6$ ]÷(W÷Ve×Vs)÷T =5.359×ΔA÷W.

2. Calculate by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 μmol of NADH in the reaction system per hour every milligram protein.

NR activity (U/mg prot)=[ΔA×Vt÷(ε×d) ×$10^6$ ]÷(Vs×Cpr)÷T =5.359×ΔA÷Cpr.

Vt: Total reaction volume, 0.0002 L;

Vs: Sample volume, 0.012 mL;

Ve: Extraction volume, 1 mL;

T: Reaction time, 0.5 h;

ε: Molar extinction coefficient of NADH: 6220 L/mol/cm;

d: Cuvette light path: 1 cm;

Cpr: Sample protein concentration, mg/mL;

W: Fresh weight of the sample, g;

$10^6$: Unit conversion factor, 1 mol = $10^6$ μmol.

b. 96 well UV flat-bottom plate

Change d(1 cm) in the formula to 0.6 cm (light path of 96 well UV plate).

**Note:**

1. When the measured absorbance value is greater than 1.5 or ΔA greater than 0.5, recommends that dilute supernatant fluid.
2. When measured value of ΔA is too small (less than 0.01), prolong the reaction time (water bath time).
3. It is not recommended to measure too many samples in one measurement to avoid delaying the enzyme reaction for too long.
4. The blank tube is a test hole for testing the quality of each reagent component. Under normal

conditions, the change of blank tube shall not exceed 0.05.

**Experimental example:**

1. Take 0.1 g Buxus microphylla and add 1 mL of Extraction reagent for homogenization, take the supernatant and dilute it twice with Extraction reagent, then operate according to the determination steps, and calculate with micro quartz colorimetric plate: $\Delta A_T = A_{T1} - A_{T2} = 1.4285-1.1089 = 0.3196$, $\Delta A_B = A_{B1} - A_{B2} = 0.8794-0.8334 = 0.046$, $\Delta A = \Delta A_T - \Delta A_B = 0.3196-0.046=0.2736$. The enzyme activity was calculated as follows:

NR activity (U/g weight) =5.359÷ΔA÷W×2(dilution ratio) =5.359×0.2736÷0.1×2=29.324 U/g weight.

2. Take 0.1 g of Yulan and add 1 mL of Extraction reagent for homogenization, take the supernatant and dilute it twice with the Extraction reagent, then operate according to the determination steps, measure and calculate with micro quartz colorimetric plate: $\Delta A_T = A_{T1} - A_{T2} = 1.4696-1.0602=0.4094$, $\Delta A_B = A_{B1} - A_{B2} = 0.8794-0.8334 = 0.046$, $\Delta A = \Delta A_T - \Delta A_B = 0.4094-0.046 = 0.3634$. The enzyme activity was calculated as follows:

NR activity (U/g weight)=5.359÷ΔA÷W×2(dilution ratio) =5.359÷0.3634÷0.1×2=38.949 U/g weight.

**Recent Product Citations:**

[1] Zhang J, Liu S, Liu CB, Zhang M, Fu XQ, Wang YL, Song T, Chao ZF, Han ML, Tian Z, Chao DY. Natural variants of molybdate transporters contribute to yield traits of soybean by affecting auxin synthesis. Curr Biol. 2023 Dec 18;33(24):5355-5367.e5. doi: 10.1016/j.cub.2023.10.072. Epub 2023 Nov 22. PMID: 37995699.

[2] Chen C, Chu Y, Huang Q, Zhang W, Ding C, Zhang J, Li B, Zhang T, Li Z, Su X. Morphological, physiological, and transcriptional responses to low nitrogen stress in Populus deltoides Marsh. clones with contrasting nitrogen use efficiency. BMC Genomics. 2021 Sep 27;22(1):697. doi: 10.1186/s12864-021- 07991-7. PMID: 34579659; PMCID: PMC8474845.

[3] Yang J, Zhang G, Peng M, Tan S, Ge S, Yang X, Liang Y, Wen Z, Xie L, Zhou T, Wu S, An J, Wang Y, Liu W, Zhang K, Zhang Z, Liu J, Shi J. Bionic Regulators Break the Ecological Niche of Pathogenic Bacteria for Modulating Dysregulated Microbiome in Colitis. Adv Mater. 2022 Sep;34(39):e2204650. doi: 10.1002/adma.202204650. Epub 2022 Aug 26. PMID: 35924734.

[4] Hou Y, Sun J, Wu B, Gao Y, Nie H, Nie Z, Quan S, Wang Y, Cao X, Li S. CPSF30-L-mediated recognition of mRNA m6A modification controls alternative polyadenylation of nitrate signaling-related gene transcripts in Arabidopsis. Mol Plant. 2021 Apr 5;14(4):688-699. doi: 10.1016/j.molp.2021.01.013. Epub 2021 Jan 27. PMID: 33515769.

[5] Amanze C, Anaman R, Wu X, Alhassan SI, Yang K, Fosua BA, Yunhui T, Yu R, Wu X, Shen L, Dolgor E, Zeng W. Heterotrophic anodic denitrification coupled with cathodic metals

recovery from on-site smelting wastewater with a bioelectrochemical system inoculated with mixed Castellaniella species. Water Res. 2023 Mar 1;231:119655. doi: 10.1016/j.watres.2023.119655. Epub 2023 Jan 23.

**References:**

[1] Bories P N, Bories C. Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase[J]. Clinical Chemistry, 1995, 41(6): 904-907.

[2] Hageman R H, Hucklesby D P. [45] Nitrate reductase from higher plants[M]//Methods in enzymology. Academic Press, 1971, 23: 491-503.

**Related Products:**

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|---|---|
| BC1500/BC1505 | Nitrate Content In Plants Assay Kit |
| BC1520/BC1525 | Ammonia Nitrogen Content In Plants Assay Kit |
| BC1480/BC1485 | Nitrite Content In Soil And Water Assay Kit |
| BC1490/BC1495 | Nitrite Content In Food Assay Kit |
| BC0070/BC0075 | Glutamate Synthase (GOGAT) Activity Assay Kit |



Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com