SUNLONG
SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Nitrate Reductase(NR) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0301

Size:50T/48S

Components:

Inducer reserve fluid: Liquid 50 mL×1. Storage at 4℃ . It should be prepared before using with 10 times dilution. Prepare the reagent when it will be used.

Extraction reagent: Liquid 80 mL×1. Storage at 4℃ .

Reagent I: Liquid 30 mL×1. Storage at -20℃ .

Reagent II: Powder ×1. Storage at -20℃ . Add 5 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℃ .

The preparation of the inducer applied liquid: Dilute the inducer reserve fluid for 10 times, for example, add 10 mL of the reagent reserve to 90 mL of distilled water and mix thoroughly.

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, which widely exists in plants and has an impact on crop yield and quality.

NR catalyzed nitrate reduction to nitrite, with $NO_3^- + NADH + H^+ \rightarrow NO_2^- + NAD^+ + H_2O$. 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.

Table centrifuge, pipettes, spectrophotometer, water-bath, 1 mL quartz cuvette, mortar/homogeniser, ice and distilled water.

Protocol

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 solution and fully grinding on ice. Centrifuge at 4000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm, set the counter to zero with distilled water.

2. Sample determination (Add reagents with the following list to the EP tube)

Reagent (μL)	Test Tube (A_T)	Blank Tube (A_B)
Sample	60	-
Extraction reagent	340	400
Reagent I	540	540
Reagent II	60	60

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: $\Delta A_T=A_{T1}-A_{T2}$, $\Delta A_B=A_{B1}-A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$

Note: Blank tubes only need to be tested 1-2 times.

III. Calculation

1. Sample weight:

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

NR (U/g fresh weight) $=[\Delta A\times Vt\div(\varepsilon\times d)\times10^6]\div(W\div Ve\times Vs)\div T=5.359\times\Delta A\div W$.

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 ofNADH in the reaction system per hour every milligram protein.

NR(U/mg prot)$=[\Delta A\times Vt\div(\varepsilon\times d)\times10^6]\div(Vs\times Cpr)\div T=5.359\times\Delta A\div Cpr$.

Vt: Total reaction volume, 0.001 L;

Vs: Sample volume, 0.06 mL;

Ve: Extraction volume, 1 mL;

T: Reaction time, 0.5 hour;

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.

Note:

1. When the measured absorbance value is greater than 1.5 or ΔA greater than 0.6, recommends that dilute supernatant fluid before measuring.

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 initial measurement of the blank tube (A_{B1}) is approximately 0.9 and the variation does 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: $\Delta A_T = A_{T1} - A_{T2} = 1.372 - 1.104 = 0.268$, $\Delta A_B = A_{B1} - A_{B2} = 0.865 - 0.827 = 0.038$, $\Delta A = \Delta A_T - \Delta A_B = 0.268 - 0.038 = 0.23$ The enzyme activity was calculated as follows

NR (U/g mass) = 5.359÷ΔA÷W×2(dilution ratio) =5.359×0.23÷0.1×2=24.6514 U / g mass.

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: $\Delta A_T = A_{T1} - A_{T2} = 1.452 - 1.066 = 0.386$, $\Delta A_B = A_{B1} - A_{B2} = 0.865 - 0.827 = 0.038$, $\Delta A = \Delta A_T - \Delta A_B = 0.4094 - 0.046 = 0.3634$

NR(U/g mass) = 5.359÷ Δ A÷W×2(dilution ratio) = 5.359×0.348÷0.1×2=37.29864 U/g mass.

Recent Product Citations:

[1] Chao Wang, Wenjing Zhang, Zehui Li,et al. FIP1 Plays an Important Role in Nitrate Signaling and Regulates CIPK8 and CIPK23 Expression in Arabidopsis. Frontier in Immunology. May 2018;(IF4.716)

[2] Fei Ding, Qiannan Hu, Meiling Wang,et al. Knockout of SlSBPASE Suppresses Carbon Assimilation and Alters Nitrogen Metabolism in Tomato Plants. International Journal of Molecular Sciences. December 2018;(IF4. 183)

[3] Li S, Tian Y, Wu K, et al. Modulating plant growth–metabolism coordination for sustainable agriculture[J]. Nature, 2018, 560(7720): 595-600.

[4] Li Z, Wang R, Gao Y, et al. The Arabidopsis CPSF30 -L gene plays an essential role in nitrate signaling and regulates the nitrate transceptor gene NRT 1.1[J]. New Phytologist, 2017, 216(4): 1205-1222.

[5] Wang C, Zhang W, Li Z, et al. FIP1 plays an important role in nitrate signaling and regulates CIPK8 and CIPK23 expression in Arabidopsis[J]. Frontiers in plant science, 2018, 9: 593.

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:

AK0428/AK0427	Nitrate Content In Plants Assay Kit
AK0426/AK0425	Ammonia Nitrogen Content In Plants Assay Kit
AK0432/AK0431	Nitrite Content In Soil And Water Assay Kit
AK0430/AK0429	Nitrite Content In Food Assay Kit
AK0600/AK0599	Glutamate Synthase (GOGAT) Assay Kit