Nitrate reductase (NR) activity Assay Kit (Griess Colorimetric Method)

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4965

Size:100T/48S

Components:

Inducer reserve fluid: Liquid 100 mL×1. Storage at 2-8℃. Dilute the inducer reserve fluid 10 times with distilled water and then use it. Just take 10 mL of inducer reserve fluid and add 90 mL of distilled water, mix well. Prepare now and use now.

Extraction reagent: Liquid 60mL×1. Storage at 2-8℃.

Reagent I: Liquid 5mL×1. Storage at -20℃.

Reagent II: Powder ×2. Storage at -20℃. Take one reagent II and add 1 mL of distilled water before using. The reagents were stored in portions at -20°C for 2 weeks. Before use, dilute the reagent II 50 times with distilled water and set aside, that is, take 10 μL of reagent II and add 490 μL of distilled water to mix.

Reagent III: Liquid 6mL×1. Storage at 2-8℃.

Reagent IV: Liquid 6mL×1. Storage at 2-8℃.

Standard: Liquid 1mL×1. Storage at 2-8℃.10 μmol/mL sodium nitrite standard solution. Before use, the standard solution will be diluted 100 times with distilled water to obtain 0.1 μmol/mL of sodium nitrite standard solution.

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^+ →NO_2^-+NAD^++H_2O. In the acidic conditions, the NO_2^- produced can participate in the diazotization reaction to produce a purple-red compound, which has an absorption peak at 540 nm, and the change in absorbance value at 540 nm indicates the enzyme activity.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, water bath/incubator, benchtop centrifuge, micro glass cuvettes/96 well plates, mortar/ homogenizer, ice and distilled water.

Protocol

I. Sample Preparation

1. Tissue pre-treatment

(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. (Induction treatments as needed, generally no induction treatment is needed, inactive pre-test results require induction treatment)

(2) According to the ratio of tissue mass (g): volume of extraction solution (mL) of 1:5~10 (weighing about 0.1 g of sample, adding 1 mL of extraction solution), grinding in an ice bath, 8000 g, centrifugation at 4°C for 10 min, and placing the supernatant on ice for measurement.

2. Cells or bacteria pre-treatment

Collect the cells or bacteria samples into the centrifuge tube, discard the supernatant, according to every 5 million cells or bacteria add 1mL of extraction solution, ultrasonic crushing bacteria or cells (power 200W, ultrasonic 3s, 10s interval, repeat 30 times). 8000g, centrifugation at 4℃ for 10min, take the supernatant, put it on the ice and wait for measurement.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 540 nm, spectrophotometer set the counter to zero with distilled water.

2. Sample determination

Reagent (μL)	Test Tube (A_T)	Control Tube (A_C)	Standard Tube (A_S)	Blank Tube (A_B)
Sample	20	20	-	-
0.1 μmol/mL standard solution	-	-	20	-
distilled water	-	75	-	95
Reagent I	75	-	75	-
Reagent II	25	25	25	25
Mix well and react at 37°C (mammals) or 25°C (other species) for 30 min				
Reagent III	50	50	50	50
Reagent IV	50	50	50	50
Mix well. The absorbance at 540 nm was measured after colour development for 20 min at room temperature and recorded as A_T, A_C, A_S and A_B, respectively.				

III. NR activity calculation

1. Calculate by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol NO_2^- in the reaction system per hour every milligram protein.

NR activity (U/mg prot) $=C_S\times(A_T-A_C)\div(A_S-A_B)\times V_S\div(V_S\times Cpr)\div T\times F=0.2\times(A_T-A_C)\div(A_S-A_B)\div Cpr\times F$

2. Calculate by Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of NO_2^- in the reaction system per hour every gram sample.

NR activity (U/ g mass) = $C_S \times (A_T-A_C) \div (A_S-A_B) \times V_S \div (W \times V_S \div V_E) \div T \times F$
$= 0.2 \times (A_T-A_C) \div (A_S-A_B) \div (A_S-A_B) \div W \times F$

3. Calculate by cell number:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of NO_2^- in the reaction system per hour every 10^4 cell number.

NR activity ($U/10^4$ cell) = $C_S \times (A_T-A_C) \div (A_S-A_B) \times V_S \div (N \times V_S \div V_E) \div T \times F = 0.2 \times (A_T-A_C) \div (A_S-A_B) \div N \times F$

C_S: Concentration of sodium nitrite standard solution,0.1 μmol/mL;

V_E: Volume of extract added,1 mL;

W: Quality of samples, g;

T: reaction time,0.5 h;

Cpr: Sample Protein Concentration, mg/mL;

V_S: Volume of sample added,0.02 mL;

N: Number of cells or bacteria,10^4 cell;

F: Sample dilution.

Note:

1. When the absorbance is greater than 1, it is recommended to dilute the sample with the extract, and note that the number of dilutions involved in the formula should be changed accordingly.
2. Add the reagents in strict accordance with the order listed in the sample determination table.

Experimental example:

1. Take 0.1 g of epipremnum aureum leaves and add 1 mL of extraction solution for homogenisation and grinding, centrifuge the supernatant and operate according to the measurement steps, measured $A_T = 0.078$, $A_C = 0.070$, $A_S = 0.268$, $A_B = 0.046$, calculate the enzyme activity according to the mass of the sample:

NR activity (U/g mass) = $0.2 \times (A_T-A_C) \div (A_S-A_B) \div W = 0.0721$ U/g mass.

2. 0.1 g of Eustoma grandiflorum leaves were homogenised and ground by adding 1 mL of extract, and the supernatant was centrifuged and operated according to the assay procedure, which resulted in $A_T = 0.088$, $A_C = 0.064$, $A_S = 0.268$, and $A_B = 0.046$, and the enzyme activity was calculated based on the mass of the samples:

NR activity (U/g mass) = $0.2 \times (A_T-A_C) \div (A_S-A_B) \div W = 0.216$ U/g mass.

Related Products:

BC0070/BC0075	Glutamate Synthase (GOGAT) Assay Kit
BC0910/BC0915	Glutamine Synthetase(GS)Activity Assay Kit
BC1500/BC1505	Nitrate Content In Plants Assay Kit

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