IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K158-S Nitrate Reductase (NR) Activity Assay Kit

Method: Colorimetric method
Specification: 100Assays **(**Can detect 48 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 1.3 U/L
Detection range: 1.3-250U/L

This manual must be read attentively and completely before using this product.

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

Application

This kit can be used to measure Nitrate Reductase (NR) activity in fresh plant tissue, culture cells, bacteria samples.

Detection significance

NR is a key enzyme of nitrogen assimilation in plant nitrogen metabolism. It is related with nitrogen absorption in crop, and can affect the yield and quality of crop. Therefore, the NR activity can be considered as one of the indexes of plant nutrition or fertilization, and it can also be used as an important indicator of varieties breeding.

Detection principle

NR can catalyze the reduction of nitrate in plants and form nitrite. The produced nitrite can form red azo compounds with p-aminobenzenesulfonic acid and α-naphthylamine under acidic conditions. The NR activity can be calculated by measuring the OD value at 540 nm.

Kits components

	Component	**Specification**	**Storage**
Reagent 1	Liquid	10 mL × 1 vial	-20℃， 3 months
Reagent 2	Liquid	5 mL × 1 vial	-20℃， 3 months
Reagent 3	Liquid	6 mL × 1 vial	2-8℃， 3 months
Reagent 4	Liquid	6 mL × 1 vial	2-8℃， 3 months，shading light
Reagent 5	Standard Stock Solution	1 mL × 1 vial	-20℃， 3 months
Preparation of 0.1 μmol/mL standard application solution: Dilute the reagent 5 with double distilled water for 100 times and mix fully.			
Reagent 6	Revulsive Stock Solution	50 mL × 1 vial	2-8℃， 3 months
Preparation of reagent 6 application solution: Dilute the reagent 6 with double distilled water for 10 times and mix fully.			
Reagent 7	Extract Solution	60 mL × 1 vial	2-8℃， 3 months

Experimental instrument

Test tube/ Microplate (96 wells), Mortar, High-precision transferpettor, Vortex mixer, Refrigerated Centrifuge, Spectrophotometer (540 nm)/ Microplate reader(540 nm)

Sample pretreatment

1. **Preparation of bacteria or cells samples:**

   Bacteria or cells: Collect the bacteria or cultured cells samples into a centrifuge tube, then centrifuge the sample and discard the supernatant. Add Extract Solution into the sediment according to the ratio of **Bacteria or Cells number (10^4): Extract Solution (mL) =500~1000: 1**(it is recommended to add 1 mL of Extract Solution into 5×10^6 bacteria or cells), then treat the sample with sonication on ice (power: 20% or 200W, 3 seconds/time, interval for 10 seconds, repeat for 30 times). Centrifuge at 8000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

2. **Preparation of tissue samples:**

   1) Add the **reagent 6 application solution** into a beaker. Take fresh plant sample, wash and dry with filter paper. Take the whole or part of the plant tissue into the inducer application solution, immerse and induce for 2 hours. Take the induced sample and dry it with filter paper. (This step is usually not required, but is required when $OD_{sample} \leq OD_{control}$)

   2) Add Extract Solution into the tissue sample according to the ratio of **Weight (g): Extract Solution (mL) =1:5~10** (it is recommended to add 1 mL of Extract Solution into 0.1 g of tissue), then homogenize the sample on ice. Centrifuge the tissue homogenate at 8000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

Operation steps

1. Preheat the Spectrophotometer or Microplate Reader for 30 min, then adjust the wavelength at 540 nm and set to zero with double distilled water.
2. Operation table

	Sample	Control	Standard	Blank
Sample (μL)	20	20		
0.1 μmol/mL standard application solution (μL)			20	
Double distilled water (μL)		75		95
Reagent 1 (μL)	75		75	
Reagent 2* (μL)	25	25	25	25
Mix fully and incubate at 37℃ (for mammal samples) or 25℃ (for samples from other species) for 30 min.				
Reagent 3 (μL)	50	50	50	50
Reagent 4 (μL)	50	50	50	50
Mix fully and incubate at 25℃ for 30 min. Measure the OD value of each well at 540 nm.				

Calculation of results

1. **Calculate according to the fresh weight of sample:**

Definition: The amount of NR in 1 g of tissue that catalyze the production of 1 μmol NO_2^- per hour is defined as 1 unit.

NR activity (μmol */h/g fresh weight*)

$$= \frac{OD_{Sample}-OD_{Control}}{OD_{Standard}-OD_{Blank}} \times \text{Concentration of standard (0.1 μmol/mL)} \times \frac{V_1}{W \times V_1 \div V_2} \div T$$

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

2. **Calculate according to the protein concentration of sample:**

Definition: The amount of NR in 1 mg of tissue protein that catalyze the production of 1 μmol NO_2^- per hour is defined as 1 unit.

NR activity (μmol */h/ mg prot*)

$$= \frac{OD_{Sample}-OD_{Control}}{OD_{Standard}-OD_{Blank}} \times \text{Concentration of standard (0.1 μmol/mL)} \times \frac{V_1}{Cpr \times V_1} \div T$$

$$= 0.2 \times \frac{OD_{Sample}-OD_{Control}}{OD_{Standard}-OD_{Blank}} \div Cpr$$

V_1: the volume of sample, 0.02 mL;
V_2: the volume of the added extract solution, 1 mL;
T: the reaction time, 0.5 h;
W: the fresh weight of sample, g;
Cpr: the concentration of protein in sample, mg/mL.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The valid of kit is 3 months.
4. Do not use components from different batches of kit.
5. NR is easily inactivated, so it must be operate quickly and perform at 4℃.
6. It is recommended to be collect the sample in fine weather to ensure the enough illumination of plant. Or apply a certain amount of nitrate nitrogenous fertilizer, which can increase the enzyme activity. The same parts of the plant should be collected.
7. The enzyme reaction should be protected from light to avoid of formation of reduced ferredoxin in chloroplast under illumination condition.
8. After the color reaction is finished, the color is stable and the colorimetric assay can be completed within 1 hour.
9. If the NR activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately.

For Research use only