Nitrite Reductase (NiR) Activity Assay Kit

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC1545

Size:100T/48S

Components:

Extract solution: Liquid 60 mL×1. Store at 2-8℃.

Reagent I: Liquid 3 mL×1. Store at 2-8℃.

Reagent II: Powder×2. Store at 2-8℃. Dissolve with 2.5mL of distilled water before use. It could be stored at 2-8℃ for two weeks.

Reagent III: Powder×1. Store at 2-8℃. Dissolve with 5mL of distilled water at 70-80℃ before use. It could be stored at 2-8℃ for three months.

Reagent IV: Liquid 10 mL×1. Store at 2-8℃.

Reagent V: Liquid 10 mL×1. Store at 2-8℃. If there is precipitation in the reagent, it could be dissolved at 70-80℃.

Standard: Liquid 1 mL×1. Store at 2-8℃. 10 μmol/mL of $NaNO_2$ standard solution.

Working solution: Reagent IV and Reagent V are mixed by the ratio of 1:1 to make working solution. Prepare when the solution will be used.

Product Description:

Nitrite reductase (NiR) is a key enzyme in the reduction of nitrite nitrogen, and plays an important role in the biogeochemical nitrogen cycle, which is widely found in microbes and plants. It catalyze the reduction of nitrite nitrogen, reduce the accumulation of nitrite nitrogen and its toxic effect on the growth and development of organisms.

Nitrite reductase can reduce NO_2^- to NO, and reduce the NO_2^- in the sample to participate in the diazotization reaction to produce a purple-red compound, that is, the change in absorbance at 540nm can reflect the activity of nitrite reductase in soil.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/ 96-well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

1. Tissue: according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant

on ice before testing.

2. Bacteria or cells: Collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. It is suggested that add 1 mL of Extraction reagent to 5 million of bacteria or cells. Use ultrasonication to split bacteria or cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 3 minutes). Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 540 nm and set spectrophotometer counter to zero with distilled water.
2. Standard working solution: dilute 10μmol/mL standard solution to 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125μmol/mL with distilled water.
3. Add reagent to a 1.5 mL EP tube:

Reagent（μL）	Matrix tube (Am)	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
Sample	-	20	20	-	-
Distilled water	20	40	-	-	-
Reagent I	40	-	40	-	-
Reagent II	40	40	40	-	-
	After mixing, react at 25℃ for 1 h.			-	-
Reagent III	40	40	40	-	-
	Fully shake for 30s, stand for 5min and take supernatant.			-	-
Superatant	70	70	70	-	-
Standard	-	-	-	70	-
Distilled water	-	-	-	-	70
Working solution	140	140	140	140	140
Mix well and stand for 5min. Measure the absorbance value at the wavelength of 540nm, and record them as Am, Ac, At, As and Ab, and calculate ΔAt = Am-(At-Ac), ΔAs = As-Ab. Each test tube should be provided with one contrast tube. Standard curve, Matrix tube (Am) and Blank tube (Ab) only be measured once or twice.					

III. Calculation:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔAt into the equation to get x (μmol/mL).

2. Calculation

1) Protein concentration:

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

NiR (U/mg prot) = x×V1÷V2÷Cpr÷T=x×7÷Cpr

2) Sample weight

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

NiR (U/g weight) = x×V1÷V2×V_E÷W÷T=x×7÷W

3) Bacteria or cells

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

NiR (U/10^4 cell) = x×V1÷V2×V_E÷N÷T=x×7÷N

V1: Total volume before taking supernatant, 0.14mL;

V2: Sample volume, 0.02 mL;

V_E: Extraction volume, 1.0 mL;

T: Reaction time, 1 hour;

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL;

N: Bacteria or cells number, 10^4.

Experimental examples:

1. Take 0.1g *poplar leaf* for sample processing and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate ΔAt = Am-(At-Ac) =0.863 0.705 0.065）） =0.223. Bring the result into the standard curve y=8.4313x+0.003 R^2=0.9999, and calculate x=0.0261. The result is calculated according to the sample mass.

NiR (U/g weight) = x×7÷W=0.0261×7÷0.1=1.827 U/g weight.

Related Products：

BC0080/BC0085	Nitrate Reductase (NR) Activity Assay Kit
BC1480/BC1485	Nitrite Assay Kit (Water And Soil)
BC1490/BC1495	Food Nitrite Content Assay Kit
BC1500/BC1505	Plant Nitrate Nitrogen Assay Kit
BC1520/BC1525	Plant Ammoniacal Nitrogen Assay Kit
BC4960/BC4965	Nitrate Reductase (NR) Activity Assay Kit (Griess-Colorimetric Method)