CheKine™ Micro Nitrate Reductase (NR) Activity Assay Kit

Cat #: KTB4016 Size: 48 T/96 T

	Micro Nitrate Reductase (NR) Activity Assay Kit		
REF	**Cat #:** KTB4016	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue, Bacteria or Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Nitrate reductase (NR, EC1.7.1.3) exists widely in plants. It is not only the key enzyme for the conversion of nitrate nitrogen to ammonia nitrogen, but also an inducing enzyme, which has an effect on the yield and quality of crops. CheKine™ Micro Nitrate Reductase (NR) Activity Assay Kit allows for the detection of nitrate reductase activity in plant tissues, bacterial, or cellular samples. The principle behind this assay involves NR catalyzing the reduction of nitrate to nitrite, following the reaction: $NO3^-$+NADH+H^+ →NO^{2-}+NAD^+ +H_2O. The resulting nitrite, under acidic conditions, then reacts quantitatively with p-amino benzenesulfonic acid and α-naphthylamine to produce a red azo compound. This red azo compound exhibits a maximum absorption peak at 540 nm, which can be measured by spectrophotometry, thereby enabling the quantification of nitrate reductase activity in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Inducer Stock Solution	25 mL	50 mL	4℃
Extraction Buffer	25 mL	50 mL	4℃
Reagent Ⅰ	5 mL	10 mL	-20℃
Reagent Ⅱ	2.5 mL	5 mL	-20℃, protected from light
Reagent Ⅲ	3 mL	6 mL	4℃
Reagent Ⅳ	3 mL	6 mL	4℃, protected from light
Reagent Ⅴ	1 mL	1 mL	-20℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Inducer Stock Solution: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃.

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Reagent III: Ready to use as supplied; Equilibrate to room temperature before use; If crystallization occurs, it should be dissolved in 60℃-90℃ water bath and used. Store at 4℃.

Reagent IV: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent V: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃.

Inducer Working Solution: Prepared before use. Dilute Inducer Stock Solution 10 times, that is, take 10 mL Inducer Stock Solution and 90 mL deionized water, mix well; Store at 4℃.

Standard: Prepared before use. Dilute Reagent Ⅴ 100 times, that is, 0.1 mL Reagent Ⅴ plus 9.9 mL deionized water, mix well; Store at -20℃.

Sample Preparation

1. Plant Tissues:

(1) Sample induction: wash the fresh specimens and suck them dry with filter paper, put them in a beaker containing Inducer Working Solution (just submerge the samples), soak for 2 h, take out the samples, and dry them with filter paper.

(2) Sample extraction: weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: It is recommended to use fresh samples without freezing. Generally, no induction treatment is required; however, if the predicted assay results show no activity ($A_{Test} \leq A_{Control}$), then induction treatment becomes necessary.

2. Bacteria or cell:

Collect 5×10^6 bacteria or cells into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or cells (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Non-induced Sample		Post-induction Sample		Blank Well (μL)	Standard Well (μL)
	Test Well (μL)	Control Well (μL)	Test Well (μL)	Control Well (μL)		
Sample	20	20	20	20	0	0
Standard	0	0	0	0	20	0
Deionized water	0	75	0	100	0	95

Reagent Ⅰ	75	0	75	0	75	0
Reagent Ⅱ	25	25	25	0	25	25
After mixing, incubate at 25℃ for 30 min.						
Reagent Ⅲ	50	50	50	50	50	50
Reagent Ⅳ	50	50	50	50	50	50
Mix well and allow to develop color at 25℃ for 20 min. Measure the optical absorbance at 540 nm for the Test Well, Control Well, Standard Well and Blank Well, which are recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.						

Note: The Standard Well and the Blank Well only need to be measured once. Set up one Control Well for each Test Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with corresponding Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

(1) Calculated by protein concentration of samples

Unit definition: One NR activity unit is defined as the amount of enzyme that catalyzes the production of 1 μmol of NO^{2-} per mg of tissue protein per hour.

$NR(U/mg\ prot)=(C_{Standard}\times V_{Sample})\times\Delta A_{Test}\div\Delta A_{Standard}\div(V_{Sample}\times Cpr)\div T=\mathbf{0.2\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

Unit definition: One NR activity unit is defined as the amount of enzyme that catalyzes the production of 1 μmol of NO^{2-} per g of fresh sample per hour.

$NR(U/g\ fresh\ weight)=(C_{Standard}\times V_{Sample})\times\Delta A_{Test}\div\Delta A_{Standard}\div(V_{Sample}\times W\div V_{Total\ Sample})\div T=\mathbf{0.2\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculated by bacteria or cell number

Unit definition: One NR activity unit is defined as the amount of enzyme that catalyzes the production of 1 μmol of NO^{2-} per 10^4 of bacteria or cells per hour.

$NR(U/g\ 10^4)=(C_{Standard}\times V_{Sample})\times\Delta A_{Test}\div\Delta A_{Standard}\div(V_{Sample}\times 500\div V_{Total\ Sample})\div T=\mathbf{0.0004\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: Standard tube concentration, 0.1 μmol/mL; V_{Sample}: Added sample volume, 0.02 mL; $V_{Total\ Sample}$: Added Extraction Buffer volume; 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 500: Total number of bacteria or cells, 10^4.

Typical Data

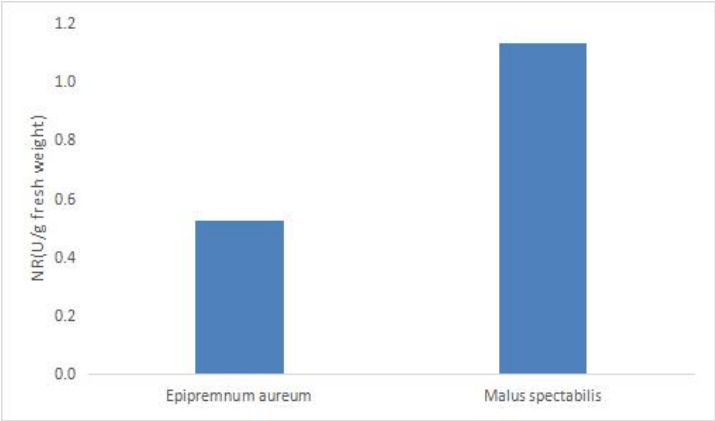

Figure 1. NR activity in Epipremnum aureum and Malus spectabilis was detected with this kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.