Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: NA0817

Size:50T/48S

Components:

Extract solution: 60 mL×1. Storage at 4℃.

Reagent I: 30 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃, protected from light. Dissolve thoroughly with 5 mL of distilled water when the solution will be used.

Reagent III: Powder×1. Storage at -20℃, protected from light. Dissolve thoroughly with 5 mL of distilled water when the solution will be used.

Reagent IV: solution×1. Storage at -20℃, protected from light. Dissolve thoroughly with 5 mL of Reagent I when the solution will be used.

Product Description:

MDHAR catalyzes MDHA to form AsA, which plays an important role in ascorbic acid redox metabolism. NADH reduces MDHA to generate AsA and NAD^+ under the conditions of MDHAR catalysis. NADH has a characteristic absorption peak at 340 nm, but NAD^+ is not. The activity of MDHAR can be calculated by measuring the decrease rate of absorption at 340 nm.

Reagents and Equipment Required but Not Provided:

Mortar/homogenizer, ice, desk centrifuge, spectrophotometer, 1 mL quartz cuvette, adjustable pipette, distilled water.

Procedure:

I. Sample preparation:

1. Tissue: Add 1 mL of Extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 10000rpm for 10 minutes at 4℃, take the supernatant and put it on ice for test.

2. Bacteria: Suggested 5-10 million with 1 mL of Extract solution. Splitting bacteria and cell with ultrasonic (ice bath, power 300W, work time 3s, interval 7s, for 3 minutes). Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and put it on ice for test.

II. Procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I at 25℃ in water bath for 30 minutes.
3. Add the following reagents to 1 mL quartz cuvette:

Reagent name (μL)	Reagent II	Reagent III	Reagent IV	Reagent I	Distilled water	Supernatant
Blank tube(B)	100	100	100	400	300	-
Test tube(T)					-	300

Mix thoroughly, detect absorbance at 340 nm at 30s and 150s, ΔA(Blank)= ΔA(B)= A1(30s)-A2(150s), ΔA(Test)=ΔA(T) =A3(30s)-A4(150s).

III. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADH in 25℃ per minute every milligram of protein.

MDHAR (U/mg prot) = [ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(Vs×Cpr)÷T

=0.268×[ΔA(T)-ΔA(B)]÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADH in 25℃ per minute every gram of sample.

MDHAR (U/g) = [ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(Vs÷Vsv×W)÷T

=0.268×[ΔA(T)-ΔA(B)]÷W

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADH in 25℃ per minute every 10^4 cell.

MDHAR (U/10^4 cell) =[ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(N÷Vsv×Vs)÷T

=0.268×[ΔA(T)-ΔA(B)]÷N

ε: NADH molar extinction coefficient, 6220 L/mol/cm;

d: Light path of cuvette, 1 cm;

10^6：1 mol=1×10^6 μmol;

Vrv: Total reaction volume,1 mL=0.001 L;

Vs: Supernate volume (mL), 0.3 mL;

Cpr: Sample protein concentration (mg/mL), need to detect separately, suggest use BCA Protein Assay Kit;

T: Reaction time (min), 2 minutes;

W: Sample weight(g);

Vsv: Extract solution volume, 1 mL;

N: Amount of cells,10^4.

Note:

1. When the determination of ΔA is greater than 0.3 it is recommended that dilute the sample or adjust the ratio of Reagent I and supernatant before the determination. For example, change 400 μL of Reagent I and 300 μL of supernatant to 600 μL of Reagent I and 100 μL of supernatant.

2. When the determination of ΔA is too small, it is recommended that the customer increase the sample or adjust the ratio of Reagent I and supernatant before the determination. For example, change 400 μL of Reagent I and 300 μL of supernatant to 200 μL of Reagent I and 500 μL of supernatant.
3. If the determination of A1 is greater than 1.5, it is recommended that dilute the sample for determination.
4. The blank tube act as the check tube hole for checking the reagent components of each tube. Under normal conditions, its OD value is about 0.5 and the change is not more than 0.01.
5. Since the extract solution contains a certain concentration of protein (about 1 mg/mL), it is necessary to subtract the protein content of the extract solution itself when determining the protein concentration of the sample.

Experimental example:

1.Take 0.1g of orange pulp and add 1 mL of Extract solution for ice bath homogenization. After centrifugation at 4℃ for 10 min at 10000 rpm, the supernatant is put on ice and operated according to the determination steps. The enzyme activity is calculated as follows: $\Delta A_T = A1_T - A2_T = 0.827-0.8=0.027$, $\Delta A_B = A1_B - A2_B = 0.536-0.532=0.004$

MDHAR (U/g mass) = $0.268\times(\Delta A_T - \Delta A_B) \times W = 0.268\times(0.027-0.004) \div 0.1 = 0.06164$ U/g mass.

Recent Product Citations：

[1] Yali Zhou,Sufang Huo,Liting Wang,et al. Exogenous 24-Epibrassinolide alleviates oxidative damage from copper stress in grape (Vitis vinifera L.) cuttings. Plant Physiology and Biochemistry. September 2018;(IF3.404)

Related Products：

NA0776/NA0535 Ascorbic Acid(AsA) Content Assay Kit
NA0775/NA0534 Dehydroascorbic Acid(DHA) Content Assay Kit