SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Monodehydroascorbate Reductase (MDHAR) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0525

Size:100T/96S

Components:

Extract solution: 110 mL×1. Storage at 4℃ .

Reagent I: 12 mL×1. Storage at 4℃ .

Reagent II: Powder×1. Storage at 4℃, protected from light. Dissolve thoroughly with 2 mL of distilled water when the solution will be used.

Reagent III: Powder×1. Storage at -20℃, protected from light. Dissolve thoroughly with 3.33 mL of distilled water when the solution will be used.

Reagent IV:liquit×1. Storage at -20℃, protected from light. Dissolve thoroughly with 2 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/microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, adjustable pipette and 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 10000 rpm for 10 minutes at 4℃, take the supernatant and put it on ice for test.

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

II. Procedure:

1. Preheat spectrophotometer/microplate for 30 minutes, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat Reagent I in water-bath at 25℃ for 30 minutes.
3. Add the following reagents to micro quartz cuvette/96 well flat-bottom UV plate:

Reagent name (μL)	Reagent II	Reagent III	Reagent IV	Reagent I	Distilled water	Supernatant
Blank tube (B)	20	20	20	80	60	-
Test tube (T)					-	60

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. Micro quartz cuvette

(1) Protein concentration:

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

MDHAR (U/mgProt) =[ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(Cpr×Vs)÷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 weight) =[ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(Vs÷Ve×W)÷T

=0.268×[ΔA(T)-ΔA(B)]÷W

(3) Cell amount:

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/10^4cell) =[ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(N×Vs÷Ve)÷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,0.2 mL=2×10^{-4}L;

Vs: Supernate volume (mL), 0.06 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 .

2. 96 well flat-bottom UV plate

Change the d- 1cm in the above formula to d-0.6cm (96 well flat-bottom UV plate) for calculation.

Note:

1. When the determination of ΔA is greater than 0.3 (greater than 0.2 when use 96 well flat-bottom UV

plate), 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(greater than 2 when use 96 well flat-bottom UV plate), 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 (about 0.3 when use 96 well flat-bottom UV plate) 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: $\Delta A_T = A1_T - A2_T = 0.8701-0.8396 = 0.0305$, $\Delta A_B = A1_B - A2_B = 0.5515-0.5474 = 0.0041$

MDHAR (U/g mass) = $0.268\times(\Delta A_T - \Delta A_B)\times W = 0.268\times(0.0305-0.0041)\times 0.1 = 0.070752$ 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:

AK0468/AK0467	Ascorbic Acid(AsA) Content Assay Kit
AK0466/AK0465	Dehydroascorbic Acid(DHA) Content Assay Kit
AK0462/AK0461	Ascorbic Acid Oxidase(AAO) Activity Assay Kit