Monodehydroascorbate Reductase (MDHAR) Activity Assay Kit

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

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: BC0655

Size:100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 12 mL×1	2-8℃
Reagent II	Powder ×1	2-8℃
Reagent III	Powder ×1	-20℃
Reagent IV	Liquid ×1	-20℃

Solution Preparation:

1. **Reagent II:** Add 2 mL distilled water before use, and dissolve it fully before use.Storage at 2-8℃.
2. **Reagent III:** Powder×1. Dissolve with 3.33 mL of distilled water one of the bottle before using.It can be stored at -20℃, after subassembly to avoid repeated freezing and thawing.
3. **Reagent IV:** Liquit×1. The liquid is placed in the EP tube inside the reagent vial. Dissolve with 2 mL of distilled water one of the bottle before using.It can be stored at -20℃, after subassembly to avoid repeated freezing and thawing.

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.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 or cell: 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. Determination 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) Calculate by sample 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/mgProt) =[ΔA(T)-ΔA(B)]÷(ε×d)×Vrv×10^6÷(Cpr×Vs)÷T

=0.268×[ΔA(T)-ΔA(B)]÷Cpr

(2) Calculate by sample mass:

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) Calculate by the number of bacteria or cells:

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) = $0.268\times(\Delta A_T - \Delta A_B) \times W = 0.268 \times(0.0305-0.0041)\times0.1 = 0.070752$ U/g mass.

Recent Product Citations:

[1] Liu N, Li J, Lv J, Yu J, Xie J, Wu Y, Tang Z. Melatonin alleviates imidacloprid phytotoxicity to cucumber (Cucumis sativus L.) through modulating redox homeostasis in plants and promoting its metabolism by enhancing glutathione dependent detoxification. Ecotoxicol Environ Saf. 2021 Jul 1;217:112248. doi: 10.1016/j.ecoenv.2021.112248. Epub 2021 Apr 23. PMID: 33901782.

[2] Yan Y, Zhao S, Ye X, Tian L, Shang S, Tie W, Zeng L, Zeng L, Yang J, Li M, Wang Y, Xie Z, Hu W. Abscisic Acid Signaling in the Regulation of Postharvest Physiological Deterioration of Sliced Cassava Tuberous Roots. J Agric Food Chem. 2022 Oct 12;70(40):12830-12840. doi: 10.1021/acs.jafc.2c05483. Epub 2022 Oct 2. PMID: 36183268.

[3] Tai F, Wang S, Liang B, Li Y, Wu J, Fan C, Hu X, Wang H, He R, Wang W. Quaternary ammonium iminofullerenes improve root growth of oxidative-stress maize through ASA-GSH cycle modulating redox homeostasis of roots and ROS-mediated root-hair elongation. J Nanobiotechnology. 2022 Jan 4;20(1):15. doi: 10.1186/s12951-021-01222-7. PMID: 34983547; PMCID: PMC8725307.

[4] Xiao S, Song W, Xing J, Su A, Zhao Y, Li C, Shi Z, Li Z, Wang S, Zhang R, Pei Y, Chen H, Zhao J. ORF355 confers enhanced salinity stress adaptability to S-type cytoplasmic male sterility maize by modulating the mitochondrial metabolic homeostasis. J Integr Plant Biol. 2023 Mar;65(3):656-673. doi: 10.1111/jipb.13382. Epub 2023 Jan 3. PMID: 36223073.

[5] Xie P, Yang Y, Gong D, Yu L, Han Y, Zong Y, Li Y, Prusky D, Bi Y. Chitooligosaccharide Maintained Cell Membrane Integrity by Regulating Reactive Oxygen Species Homeostasis at Wounds of Potato Tubers during Healing. Antioxidants (Basel). 2022 Sep 10;11(9):1791. doi: 10.3390/antiox11091791. PMID: 36139864; PMCID: PMC9495885.

Related Products:

BC1230/BC1235	Ascorbic Acid(AsA) Content Assay Kit
BC1240/BC1245	Dehydroascorbic Acid(DHA) Content Assay Kit
BC1260/BC1265	Ascorbic Acid Oxidase(AAO) Activity Assay Kit

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