Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0650

Size:50T/48S

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 60 mL×1	2-8℃
Reagent I	Liquid 30 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 5 mL distilled water before use, and dissolve it fully before use.Storage at 2-8℃.
2. **Reagent III:** Powder×1. Dissolve with 5 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 5 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, 1 mL quartz cuvette, adjustable pipette, 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 10000rpm

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for 10 minutes at 4°C, take the supernatant and put it on ice for test.

2. Bacteria or cell: 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°C, take the supernatant and put it on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I at 25°C 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. 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°C per minute every milligram of protein.

$$MDHAR\ (U/mg\ prot) = [\Delta A(T)-\Delta A(B)]\div(\varepsilon\times d)\times Vrv\times 10^6\div(Vs\times Cpr)\div T$$
$$=0.268\times[\Delta A(T)-\Delta A(B)]\div Cpr$$

(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°C per minute every gram of sample.

$$MDHAR\ (U/g) = [\Delta A(T)-\Delta A(B)]\div(\varepsilon\times d)\times Vrv\times 10^6\div(Vs\div Vsv\times W)\div T$$
$$=0.268\times[\Delta A(T)-\Delta A(B)]\div W$$

(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°C per minute every 10^4 cell.

$$MDHAR\ (U/10^4\ cell) =[\Delta A(T)-\Delta A(B)]\div(\varepsilon\times d)\times Vrv\times 10^6\div(N\div Vsv\times Vs)\div T$$
$$=0.268\times[\Delta A(T)-\Delta A(B)]\div N$$

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] Jia Y, Yin X, Zhao J, Pan Y, Jiang B, Liu Q, Li Y, Li Z. Effects of 24-Epibrassinolide, melatonin and their combined effect on cadmium tolerance in Primula forbesii Franch. Ecotoxicol Environ Saf. 2023 Jul 3;262:115217. doi: 10.1016/j.ecoenv.2023.115217. Epub ahead of print. PMID: 37406607.

[2] Lin D, Yan R, Xing M, Liao S, Chen J, Gan Z. Fucoidan treatment alleviates chilling injury

in cucumber by regulating ROS homeostasis and energy metabolism. Front Plant Sci. 2022 Dec 23;13:1107687. doi: 10.3389/fpls.2022.1107687. PMID: 36618644; PMCID: PMC9816408.

[3] Lu X, Chen G, Ma L, Zhang C, Yan H, Bao J, Nai G, Wang W, Chen B, Ma S, Li S. Integrated

transcriptome and metabolome analysis reveals antioxidant machinery in grapevine exposed to salt and alkali stress. Physiol Plant. 2023 May-Jun;175(3):e13950. doi: 10.1111/ppl.13950. PMID: 37291799.

[4] Li Y, Niu L, Zhou X, Liu H, Tai F, Wang W. Modifying the Expression of Cysteine Protease Gene PCP Affects Pollen Development, Germination and Plant Drought Tolerance in Maize. Int J Mol Sci. 2023 Apr 17;24(8):7406. doi: 10.3390/ijms24087406. PMID: 37108569; PMCID: PMC10138719.

[5] Jia L, Li Y, Liu G, He J. UV-C delays senescence in 'Lingwu long' jujube fruit by regulating ROS and phenylpropanoid metabolism. Plant Physiol Biochem. 2023 Jan;194:383-393. doi: 10.1016/j.plaphy.2022.11.030. Epub 2022 Nov 26. PMID: 36473328.

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BC1240/BC1245 Dehydroascorbic Acid(DHA) Content Assay Kit

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