Dehydroascorbate Reductase(DHAR) Activity Assay Kit

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: BC0665

Size:100T/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 110 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II	Powder ×1	-20℃
Reagent III	Powder ×1	2-8℃
Reagent IV	Liquid 8 mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve with 3 mL of distilled water one of the bottle before using, and unused liquid can be stored at -20℃ for 2 weeks.

2. Reagent III: The powder is placed in the EP tube inside the reagent vial. Dissolve with 3.5 mL of distilled water one of the bottle before using, and unused liquid can be stored at 2-8℃.

3. Standard: powder ×1 bottle, add 1 mL of distilled water before use. to prepare a standard solution of 10 mg/mL..

Product Description:

Dehydroascorbate reductase (DHAR) is an important antioxidant enzyme in plants and a key enzyme that promotes ascorbic acid regeneration in the ascorbate-glutathione oxidation cycle. In the circulation DHAR maintain the normal metabolic level of ascorbic acid in plants through ascorbic acid, and plays an important role in protecting cellular components from oxidative damage.

DHAR catalyzes the reduction of dehydroascorbic acid (DHA) by reducing glutathione (GSH) to produce AsA. GSH can react with 5,5'-dithio-bis- (2-nitrobenzoic acid) (DTNB) to produce 2-Nitro-5-mercaptobenzoic acid (TNB) and glutathione disulfide (GSSG). TNB has maximum light absorption at a wavelength of 412 nm. DHAR activity is calculated by measuring the reduction rate of GSH.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection

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Reagents and Equipment Required but Not Provided:

Low temperature centrifuge, spectrophotometer/microplate reader, water bath, mortar/homogenizer, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, ice and distilled water.

Operation procedure:

I. Sample Extraction:

1. Tissue sample:

   According to the mass of the tissue (g): the volume of the Extract solution (mL) is 1: 5 ~ 10. Suggested 0.1g of tissue with 1 mL of Extract solution. Fully grind on ice, centrifuge at 8000g and 4℃ for 10 min. Supernatant is placed on ice for test.

2. Bacteria or cells:

   According to the number of cells (10^4): the volume of the Extract solution (mL) is 500 ~ 1000: 1. Suggest 5 million with 1 mL of Extract Solution. Use ultrasonic to split bacteria or cells (power 300W, work time 3s，interval 7s，total time 3 min). centrifuge at 8000g and 4℃ for 10 min. Supernatant is placed on ice for test.

3. Serum and other liquids: direct detection.

II. Determination procedure:

1 Preheat the spectrophotometer/microplate reader 30min, adjust wavelength to 412nm, set zero with distilled water.

2 Preparation of standard solution: Dilute 10 mg / mL standard solution with distilled water to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 mg / mL standard solution for future use.

3 Add reagents with the following list:

Reagent name (μL)	Test tube (T)	Control tube(C)	Blank tube (B)	Control tube of Blank (CB)	Standard tube(S)	Blank tube of Standard(BS)
Sample	20	20	-	-	-	-
Standard solution	-	-	-	-	20	
Distilled water	-	-	-	-	-	20
Reagent I	100	140	120	160	140	140
Reagent II	20	-	20	-	-	-
Reagent III	20	-	20	-	-	-
Reagent IV	40	40	40	40	40	40

Mix well, and measure the absorbance at 412 nm of each tube after standing at 25°C for 20 minutes, and record them as A_T and A_C, A_B, A_{CB}, A_S and A_{BS}. $\Delta A = (A_B-A_{CB})-(A_T-A_C)$, $\Delta A_S= A_S-A_{BS}$. The blank tube, control tube of blank, standard tube and blank tube of standard need only be tested 1-2 times.

III. Calculation of DHAR activity:

1 Drawing of standard curve:

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA into the equation

to get x (mg/ mL).

2 Calculated of DHAR activity.

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 μg of GSH every milligram of tissue protein per minute.

DHAR activity (U/mg prot) = $x \times V_E \div (V_E \times Cpr) \times 10^3 \div T = 50x \div Cpr$

2) Calculate by sample mass:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μg of GSH every gram of tissue per minute.

DHAR activity (U/g fresh weight) = $x \times V_E \div W \times 10^3 \div T = 50x \div W$

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 μg of GSH every 10 thousand bacteria or cells per minute.

DHAR activity (U/10^4 cell) = $x \times V_E \div N (10^4) \times 10^3 \div W \div T = 50x \div N (10^4)$

4) Calculated by serum and other liquids:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μg of GSH every milliliter of liquids per minute.

DHAR activity (U/mL) = $x \times V_S \div V_S \times 10^3 \div T = 50x$

V_E: volume of extraction solution, 1 mL;

10^3: unit conversion factor, 1mg = 10^3 μg;

Cpr: sample protein concentration, mg / mL, protein concentration determined by itself;

W: sample mass, g;

T: reaction time: 20 min;

V_S: Add sample volume, 0.02 mL.

N: Number of cell.

Experimental example:

Take 0.1g of Phytolacca acinosa and add 1ml extract, grind the homogenate on ice, 8000 g, centrifuge at 4℃ for 10 minutes. The supernatant is put on ice, and the operation is performed according to the determination steps. measured by 96 well plate: $\Delta A = (A_B - A_{BC}) - (A_T - A_C)$ = (1.169-0.088) - (0.974-0.357) = 0.464, the standard curve y = 3.2972x+0.0011, and x =0.1404 mg/mL is calculated according to the standard curve

DHAR (U/g mass) = 50x÷W = 70.2 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] Zou Y, Cao S, Zhao B, Sun Z, Liu L, Ji M. Increase in glutathione S-transferase activity and antioxidant damage ability drive resistance to bensulfuron-methyl in Sagittaria trifolia. Plant Physiol Biochem. 2022 Nov 1;190:240-247. doi: 10.1016/j.plaphy.2022.09.007. Epub 2022 Sep 15. PMID: 36148723.

[3] Gao F, Shi Y, Wang R, Tretyakova IN, Nosov AM, Shen H, Yang L. Exogenous Glutathione Promotes the Proliferation of Pinus koraiensis Embryonic Cells and the Synthesis of Glutathione and Ascorbic Acid. Plants (Basel). 2022 Sep 30;11(19):2586. doi: 10.3390/plants11192586. PMID: 36235452; PMCID: PMC9571378.

[4] 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.

[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:

BC0650/BC0655	Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit
BC1230/BC1235	Ascorbic Acid(AsA) Content Assay Kit
BC1240/BC1245	Dehydroascorbic Acid(DHA) Content Assay Kit

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