Dehydroascorbate Reductase (DHAR) Activity Assay Kit

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

Detection instrument: Spectrophotometer

Catalog Number: BC0660

Size: 50T/24S

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	-20℃
Reagent III	Powder ×1	2-8℃
Reagent IV	Liquid 10 mL×1	2-8℃
Standard	Powder ×1 bottle	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.

Reagents and Equipment Required but Not Provided:

Low temperature centrifuge, spectrophotometer, water bath, mortar/homogenizer, 1 mL glass cuvette, adjustable pipette, ice 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 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 1mL 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 1mL 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 10min. Supernatant is placed on ice for test.

3. Serum and other liquids: direct detection.

II. Determination procedure:

1 Preheat the spectrophotometer 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	50	50	-	-	-	-
Standard solution	-	-	-	-	50	
Distilled water	-	-	-	-	-	50
Reagent 1	250	350	300	400	350	350
Reagent 2	50	-	50	-	-	-
Reagent 3	50	-	50	-	-	-
Reagent 4	100	100	100	100	100	100
Distilled water	500	500	500	500	500	500
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, 0.05 mL.

N: Number of cell

Experimental example:

Take 0.1 g of Phytolacca acinosa and add 1 mL of Extract solution, grind the homogenate on ice, centrifuge at 4℃ and 8000g for 10 minutes The supernatant is placed on ice and operated according to the determination steps. The measured and calculated $\Delta A = (A_B - A_{CB}) - (A_T - A_C) = (0.905-0.045) - (0.896-0.292) = 0.256$, the standard curve: y = 2.153x + 0.0089, the standard curve x = 0.1148 mg/mL

DHAR (U/g mass) = 50x ÷ W = 57.4 U/g mass.

Recent Product Citations:

[1] 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.

[2] Zhang Z, Zhang Y, Yuan L, Zhou F, Gao Y, Kang Z, Li T, Hu X. Exogenous 5-aminolevulinic acid alleviates low-temperature injury by regulating glutathione metabolism and β-alanine metabolism in tomato seedling roots. Ecotoxicol Environ Saf. 2022 Oct 15;245:114112. doi: 10.1016/j.ecoenv.2022.114112. Epub 2022 Sep 22. PMID: 36155340.

[3] 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.

[4] 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.

[5] 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.

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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