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

Dehydroascorbate Reductase(DHAR) Activity Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: AK0277

Size: 100T/48S

Components:

Extract solution: 110 mL × 1, stored at 4°C.

Reagent 1: 20 mL × 1, stored at 4°C.

Reagent 2: Powder × 1, stored at -20°C. Just before use, add 3 mL of distilled water to fully dissolve. Unused reagents are stored at -20°C for 2 weeks after dispensing.

Reagent 3: powder × 1, stored at 4 °C. Just before use, add 3.5 mL of distilled water to fully dissolve.

Reagent 4: 8 mL × 1, stored at 4°C.

Standard: powder 10 mg × 1, stored at 4°C. Just before use, add 1 mL of distilled water 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.

Required material

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

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 4C 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 1	100	140	120	160	140	140
Reagent 2	20	-	20	-	-	-
Reagent 3	20	-	20	-	-	-
Reagent 4	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) Calculated by 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) Calculated by sample weight

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) Calculated by bacteria or cell amount:

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×V_E÷N （10^4） ×10^3÷W÷T =50x÷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 ×V_S ÷V_S × 10^3 ÷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:

1. 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: ΔA = (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.

Related Products：

AK0526/AK0525	Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit
AK0468/AK0467	Ascorbic Acid(AsA) Content Assay Kit
AK0466/AK0465	Dehydroascorbic Acid(DHA) Content Assay Kit