

# Dehydroascorbate Reductase (DHAR) Activity Assay Kit

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

**Detection instrument:** Spectrophotometer

**Cat No:** BC0660

**Size:** 50T/24S

## Components:

Extract solution: 60 mL × 1, stored at 4°C.

Reagent 1: 30 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: 10 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, water bath, mortar/homogenizer, 1 mL glass cuvette, 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 1mL of Extract solution. Fully grind on ice, centrifuge at 8000g and 4°C 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°C 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 ( $\mu\text{L}$ )	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  $\Delta A_S$  as the y-axis, draw a standard curve to get the standard equation  $y = kx + b$ , and bring  $\Delta A$  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  $\mu\text{g}$  of GSH every milligram of tissue protein per minute.

$$\text{DHAR activity (U/mg prot)} = x \times V_E \div (V_E \times C_{pr}) \times 10^3 \div T = 50x \div C_{pr}$$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1  $\mu\text{g}$  of GSH every gram of tissue per minute.

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

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  $\mu\text{g}$  of GSH every 10 thousand bacteria or cells per minute.

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

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  $\mu\text{g}$  of GSH every milliliter of liquids per minute.

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

$V_E$ : volume of extraction solution, 1 mL;

$10^3$ : unit conversion factor, 1mg =  $10^3$   $\mu\text{g}$ ;

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°C 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

$$\text{DHAR (U/g mass)} = 50x \div W = 57.4 \text{ U/g mass.}$$

**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