



Dehydroascorbate Reductase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1054

(Version 2.5D)

Detection and Quantification of Dehydroascorbate Reductase (DHAR)
Activity in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Plant dehydroascorbate reductase (DHAR) is a physiologically important reducing enzyme in the ascorbate-glutathione recycling reaction. DHA must be converted to AsA by DHAR in the presence of glutathione (GSH) as a reducing agent. Thus, DHAR is a key factor in maintaining a reduced AsA level in the adaptation to environmental conditions.

Dehydroascorbate Reductase Activity Microplate Assay Kit provides a simple and direct procedure for measuring dehydroascorbate reductase activity in a variety of samples. In this kit, dehydroascorbate reductase catalyzes reduction of dehydroascorbic acid to ascorbic acid, and the intermediate reacts with the dye reagent. The rate of decrease in the absorbency at 450 nm, is a measure of DHAR activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	1 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Substrate Diluent before use, vortex until all substrate dissolves completely. Prepare for immediate use or store at -20°C for 3-4 days after reconstitution.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Prepare for immediate use or store in dark at -20°C for 2-3 days after reconstitution.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix, store at 4°C. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 16000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 16000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	70 μ l	70 μ l	--	--
Sample	10 μ l	--	--	--
Standard	--	--	100 μ l	--
Distilled water	--	10 μ l	--	100 μ l
Substrate	10 μ l	10 μ l	--	--
Coenzyme	10 μ l	10 μ l	--	--
Dye Reagent A	90 μ l	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l
Mix, keep in dark for 5 minutes at room temperature, record absorbance measured at 450 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of DHAR activity is defined as the enzyme converts 1 μmol NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mg})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

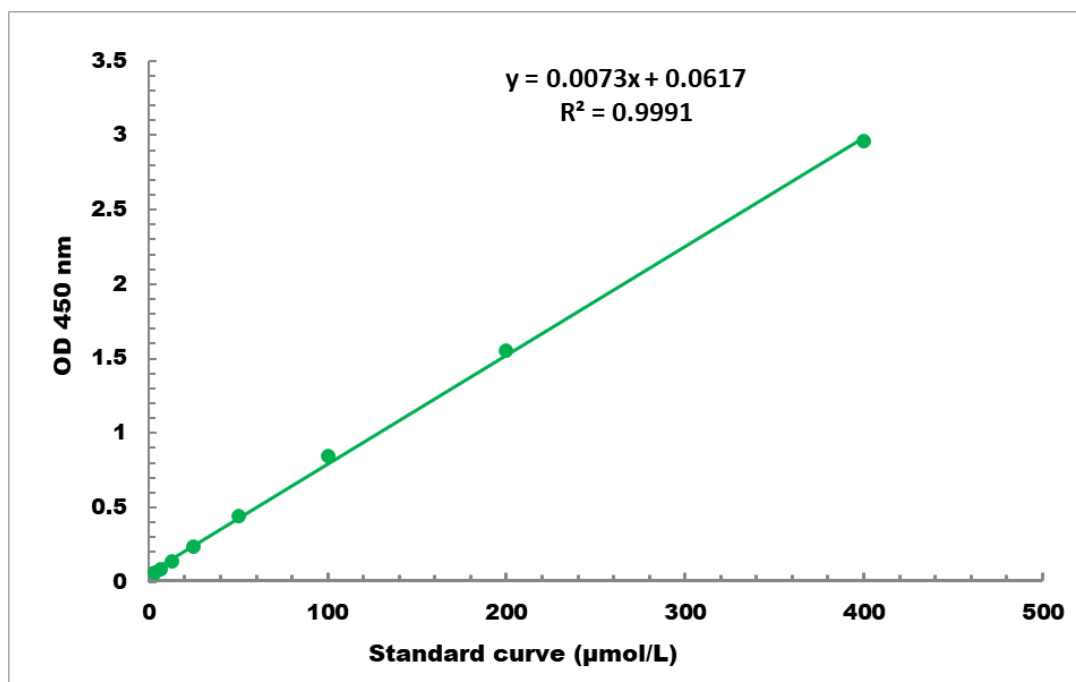
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4µmol/L - 400 µmol/L