# Dehydroascorbate Reductase

# Colorimetric Microplate Assay Kit

# User Manual

**Catalog # CAK1054**

(Version 2.4D)

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

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

## I. INTRODUCTION

Plant dehydroascorbate reductase (DHAR) is a physiologically important reducing enzyme in the ascorbateglutathione 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 Microplate Assay Kit provides a simple and direct procedure for measuring dehydroascorbate reductase activity in a variety of samples. In this kit, dehydroascorbate reductase catalyzed reduction of dehydroascorbic acid to ascorbic acid, and the intermediate react 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 | |

**Note:**

**Substrate**: add 1 ml Substrate Diluent before use, vortex until all substrate dissolve completely.

**Coenzyme**: add 1 ml Reaction Buffer to dissolve before use, mix, store at -20°C.

**Dye Reagent A**: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

**Standard**: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

## 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. 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 $5 \times 10^6$ 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.

## V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

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

**Note:**

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

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

1. According to the protein concentration of sample

$$DHAR\ (U/mg/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.8 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$DHAR\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$DHAR\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$DHAR\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.8 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml

$V_{Sample}$: the volume of sample, 0.01 ml

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

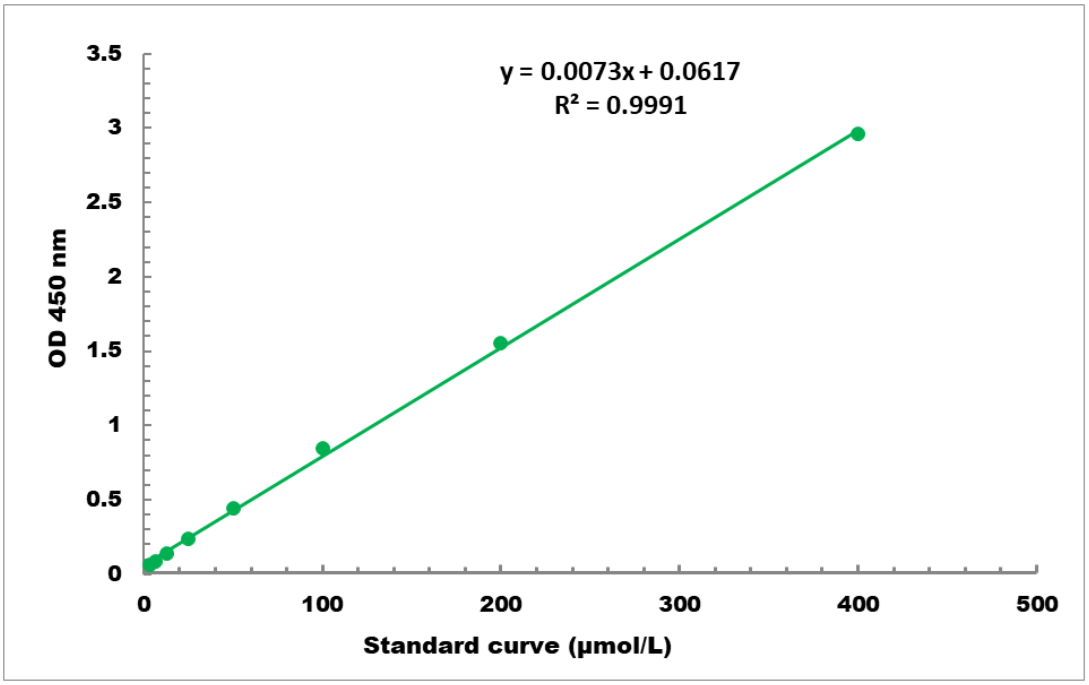
N: the quantity of cell or bacteria, N × $10^4$

$V_{Assay}$: the volume of Assay buffer, 1 ml

T: the reaction time, 5 minutes

## VII. TYPICAL DATA

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




Detection Range: 4 μmol/L - 400 μmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES