

Monodehydroascorbate Reductase Microplate Assay Kit

Basic information:

Catalog No.: UAK1053 Size: 100 Assays

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

I. INTRODUCTION

In plants, the monodehydroascorbate reductase (MDAR) is an enzymatic component of the glutathione-ascorbate cycle that is one of the major antioxidant systems of plant cells for the protection against the damages produced by reactive oxygen species (ROS). The MDAR activity has been described in several cell compartments, such as chloroplasts, cytosol, mitochondria, glyoxysomes, and leaf peroxisomes.

The assay is initiated with the enzymatic catalysis of the NADH by MDAR. NADH can be measured at a colorimetric readout at 340 nm.

II. KIT COMPONENTS

| Component | Volume | Storage |
|--------------------|------------|----------------------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30ml x 4 | 4 °C |
| Reaction Buffer | 20 ml x 1 | 4 °C |
| Enzyme | Powder x 1 | -20 °C, keep in dark |
| Substrate | Powder x 1 | -20 °C |
| Standard | Powder x 1 | -20 °C |
| Technical Manual | 1 Manual | |

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Substrate: add 1 ml Reaction Buffer to dissolve before use.

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 $\mu\text{mol/L}$.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. 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 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 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 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

| Reagent | Sample | Standard | Blank |
|-----------------|-------------------|----------|-------|
| Reaction Buffer | 170 μl | -- | -- |

| | | | |
|--|-------|--------|--------|
| Enzyme | 10 µl | -- | -- |
| Substrate | 10 µl | -- | -- |
| Mix. | | | |
| Standard | -- | 200 µl | -- |
| Distilled water | -- | -- | 200 µl |
| Sample | 10 µl | -- | -- |
| Mix, measured at 340 nm and record the absorbance of 10th second and 130th second. | | | |

Note: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.

VI. CALCULATION

Unit Definition: One unit of MDAR is defined as the enzyme oxidize 1 nmol NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{MDAR (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{MDAR (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{MDAR (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{MDAR (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (OD_{\text{Sample}(10\text{S})} - OD_{\text{Sample}(130\text{S})}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, 400 $\mu\text{mol/L}$ = 400 nmol/ml;

V_{Standard} : the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

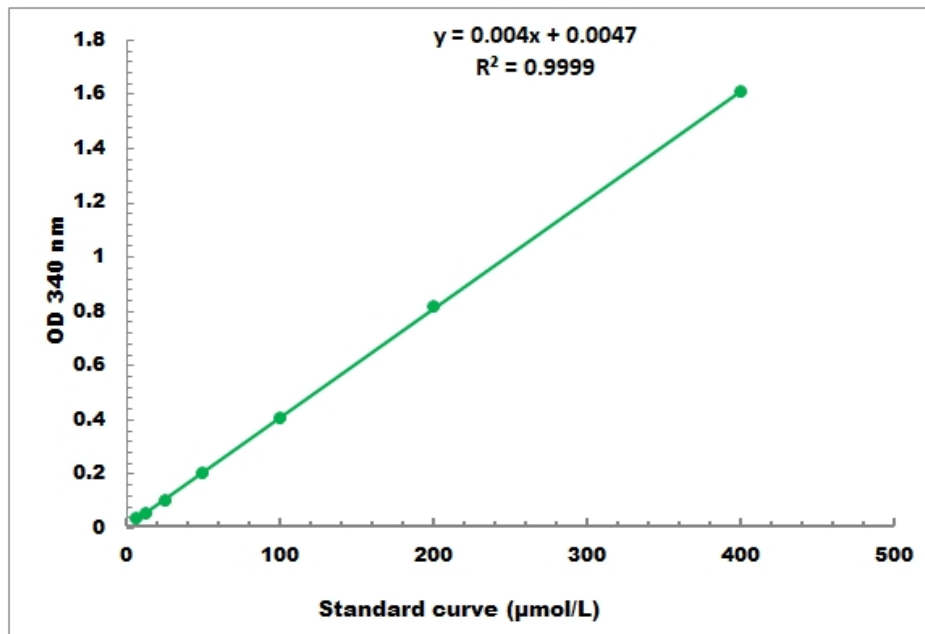
V_{Sample} : the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

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



Detection Range: 4 $\mu\text{mol/L}$ - 400 $\mu\text{mol/L}$