Monodehydroascorbate Reductase Microplate Assay Kit

Catalog # AS0053

Detection and Quantification of Monodehydroascorbate Reductase Activity in Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powderx 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 Bufferto dissolve before use.

Substrate: add 1 ml Reaction Bufferto dissolve before use.

Standard:add 1 mldistilled water to dissolve before use; then add 0.2 ml into 0.8 mldistilled water, the concentration will be 400 µmol/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 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm all reagentsto 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 recordthe absorbance of 10thsecond 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 MDARis defined asthe enzymeoxidize 1nmolNADH per minute.

1. According to the protein concentration of sample

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

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

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

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$MDAR\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400nmol/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 ×10^4;

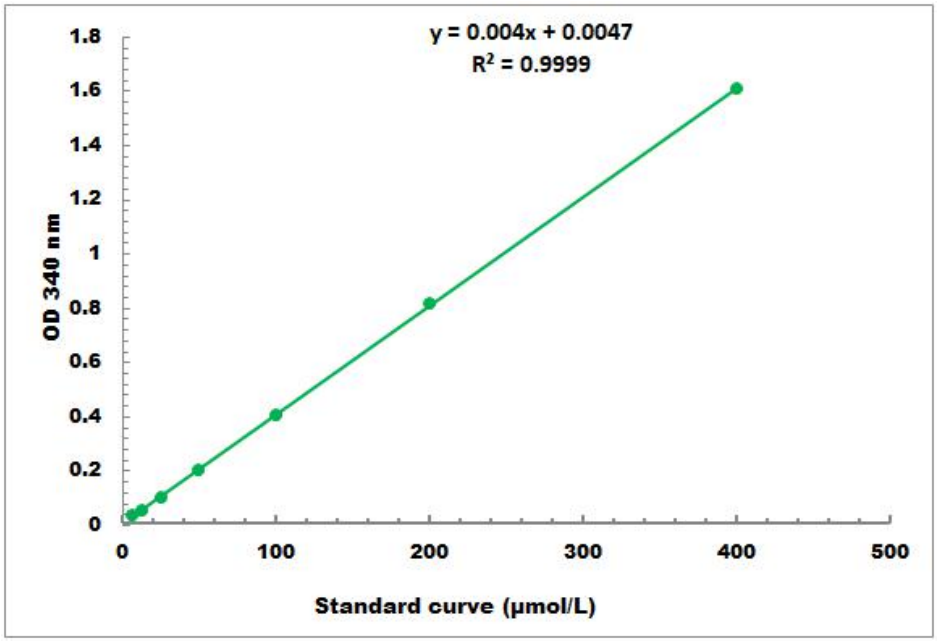
V_{Sample}: the volume of sample, 0.01 ml;

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: 4μmol/L - 400 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES