



Dehydroascorbic Acid Microplate Assay Kit User Manual

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Detection and Quantification of Dehydroascorbic Acid (DHA)

Content in Tissue extracts, Cell lysate Samples.

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

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I. INTRODUCTION

Dehydroascorbic acid (DHA) is an oxidized form of ascorbic acid (vitamin C). It is actively imported into the endoplasmic reticulum of cells via glucose transporters. The assay is initiated with the reduction reaction of the DHA by DTT. The reduction reaction products AsA can be measured at a colorimetric readout at 265 nm.



II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme Diluent	18 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
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Note:

Enzyme working solution: add 1 ml Enzyme Diluent into the Enzyme tube, mix. Then transfer all the enzyme into the Enzyme Diluent bottle, mix. The Enzyme working solution must be prepared before use.

Standard: add 10 ml distilled water into the bottle, mix; then add 250 µl standard into 750 µl distilled water. The concentration of the standard will be 500 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 265 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 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 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 the Enzyme working solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard
Sample	20 μ l	--
Standard	--	20 μ l
Enzyme working solution	180 μ l	180 μ l

Mix, measured at 265 nm and record the absorbance of the 10th second and the 130th second.

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned} \text{DHA } (\mu\text{mol}/\text{mg}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \\ &\quad \text{OD}_{\text{Standard}(10\text{S})}) \times V_{\text{Standard}} / (C_{\text{Protein}} \times V_{\text{Sample}}) \\ &= 0.5 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \text{OD}_{\text{Standard}(10\text{S})}) / \\ &\quad C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{DHA } (\mu\text{mol}/\text{g}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \text{OD}_{\text{Standard}(10\text{S})}) \\ &\quad \times V_{\text{Standard}} / (W \times V_{\text{Sample}} / V_{\text{Total}}) \\ &= 0.5 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \text{OD}_{\text{Standard}(10\text{S})}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{DHA } (\mu\text{mol} / 10^4) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \\ &\quad \text{OD}_{\text{Standard}(10\text{S})}) \times V_{\text{Standard}} / (N \times V_{\text{Sample}} / V_{\text{Total}}) \\ &= 0.5 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}(130\text{S})} - \text{OD}_{\text{Standard}(10\text{S})}) / N \end{aligned}$$

C_{Standard} : the protein concentration, 500 $\mu\text{mol}/\text{L}$ = 0.5 $\mu\text{mol}/\text{ml}$;

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

W: the weight of sample, g;

V_{Sample} : the volume of sample, 0.02 ml;

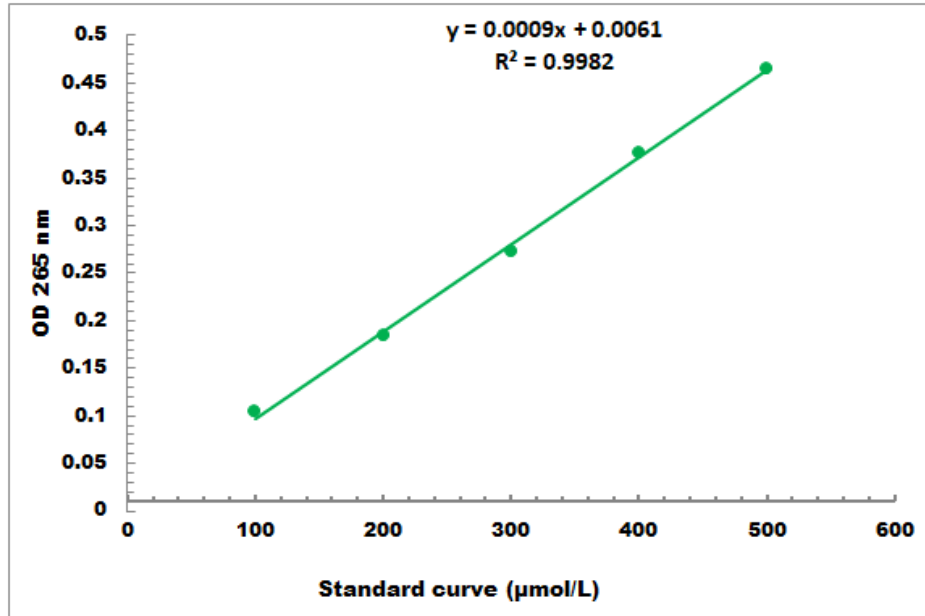
V_{Standard} : the volume of sample, 0.02 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 50 µmol/L - 500 µmol/L