Dehydroascorbic Acid

Microplate Assay Kit

User Manual

Catalog # CAK1049

(Version 1.2D)

Detection and Quantification of Dehydroascorbic Acid (DHA)

Content in Tissue extracts, Cell lysate Samples.

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

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
Technical Manual	1 Manual	

Note:

Enzyme working solution: add 1 ml Enzyme Diluent into the Emzyme tube, mix. Then transfer all the emzyme 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, 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 5×10^6 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 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 µl	--
Standard	--	20 µl
Enzyme working solution	180 µl	180 µl
Mix, measured at 265 nm and record the absorbance of the 10th second and the 130th second.		

Note:

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

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

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

VI. CALCULATION

1. According to the protein concentration of sample

$$DHA\ (\mu mol/mg) = C_{Standard} \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) \times V_{Standard} / (C_{Protein} \times V_{Sample})$$

$$= 0.5 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) / C_{Protein}$$

2. According to the weight of sample

$$DHA\ (\mu mol/g) = C_{Standard} \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) \times V_{Standard} / (W \times V_{Sample} / V_{Total})$$

$$= 0.5 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) / W$$

3. According to the quantity of cells or bacteria

$$DHA\ (\mu mol/10^4) = C_{Standard} \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) \times V_{Standard} / (N \times V_{Sample} / V_{Total})$$

$$= 0.5 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard(10S)}) / N$$

$C_{Standard}$: the protein concentration, 500 μmol/L = 0.5 μmol/ml;

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

W: the weight of sample, g;

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

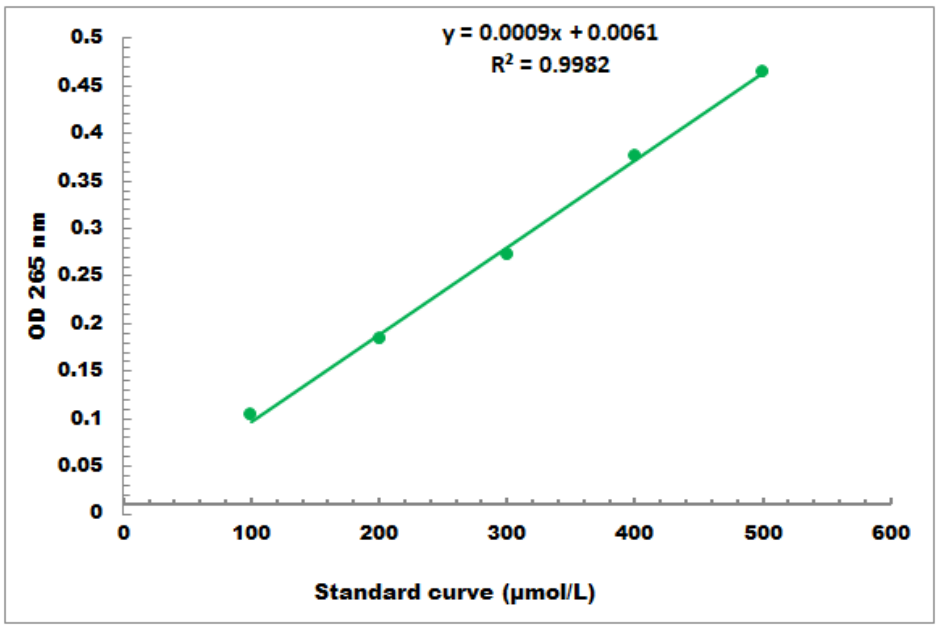
$V_{Standard}$: the volume of sample, 0.02 ml;

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

N: the quantity of cell or bacteria, N × 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

VIII. TECHNICAL SUPPORT

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

IX. NOTES