Dehydroascorbic Acid

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1049

(Version 1.3D)

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. Dehydroascorbic Acid Colorimetric Microplate Assay Kit provides a convenient means tomeasure dehydroascorbic acidconcentration in biological samples.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 mlx 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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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. REAGENT PREPARATION

Standard: Dissolvein10 ml distilled waterto generate 2 mmol/Lstandardsolution,store at -20 °C for 2-3 days.Then dilute to500 µmol/Lstandard top solutionby adding250 µlstock solution into 750 µl distilled water. Performserial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be 500/400/300/200/100/50µmol/L. Prepare standard solutionfor immediateuse.

Enzyme Working Solution: Briefly centrifuge Enzyme prior to opening.Add 1 ml Enzyme Diluent into the Enzyme tube, mix. Then transfer all the enzyme into the Enzyme Diluentbottle, mix. The EnzymeWorking Solutionmust be prepared before use.

V. 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%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 20minutes, 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 mlAssay Buffer on ice, centrifuged at 10000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm theEnzyme working solution to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Assay Buffer	--	--	20 µl
Enzyme Working Solution	180 µl	180 µl	180 µl
Mix,measured at 265 nm and recordthe absorbance of the 10th second and the 130th second.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times\frac{V_{Standard}}{V_{Sample}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^4)$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

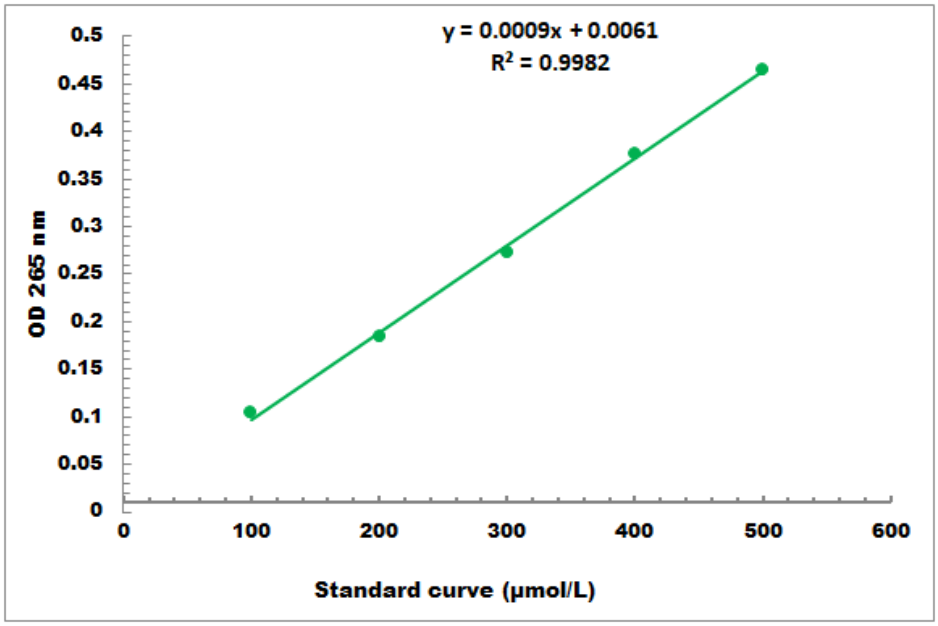
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

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

VIII. 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