Ascorbic Acid

Colorimetric MicroplateAssay Kit

User Manual

Catalog # CAK1048

(Version 3.1E)

Detection and Quantification of Ascorbic Acid (AsA) Content in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Ascorbic Acid, also known as Vitamin C, is a six-carbon lactone produced by plants and some animal species but not by humans and other primates. Ascorbic acid functions as an enzymatic cofactor for multiple enzymes, serving as an electron donor for monooxygenases and dioxygenases. Ascorbic acid also functions as a powerful antioxidant, particularly in regards to reactive oxygen species.

Ascorbic AcidColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of ascorbic acid concentration in a variety of samples.The addition of enzyme to parallel samples removes ascorbic acid specifically. Then, the probe is reduced to ferrous ion ($Fe2^{+}$) by total or residualantioxidant, which reacts with dye reagent to form a colored product. The color intensity reduction after ascorbic acid removal at 510nm is proportional to the ascorbic acid concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer A	16 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	4 °C
Reaction Buffer B	16 ml x 1	4 °C
Dye Reagent	Powder x 2	4 °C, keep in dark
Standard	Powder x 2	4 °C, keep in dark
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolveeach vial in0.25 ml distilled waterto generate 50 mmol/L of Stock Solution.Then dilute to 2 mmol/L top standardsolutionby adding 40 µl Stock Solution to 960µl distilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 2.0/1.0/0.5/0.25/0.125/0.0625/0.0312mmol/L.Keep in dark and prepare standard curvefresh for immediate use.

Enzyme: Briefly centrifuge prior to opening. Dissolvein1 ml distilled water before use.Store at -80 °C for 1 month.

Probe: Briefly centrifuge prior to opening. Dissolvein1 ml distilled water to generate probe stock solution.Dilute the stock solution 5-fold using distilled water to prepare the probeworking solution (eg. 200 µlto 800 µldistilled water). Store at -20 °C for 1 monthor 4°C for 3 days.

Dye Reagent: Briefly centrifuge prior to opening. Dissolveeach vialin1 ml distilled water before use.Keep in dark and store at -20 °C for 1 monthor 4°C for 3 days.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

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 (the quantity should be adjusted according to the actual situation of the sample), sonicate (with power 20%, sonicate 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

Weighout 0.1 g tissue (the quantity should be adjusted according to the actual situation of the sample), homogenize with 1 mlAssay 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.

3.For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add samples to a paired set of wells in a 96-well plate as Sample and Control.

Add following reagents intothe microplate:

Reagent*	Sample**	Control**	Standard	Blank
Reaction Buffer A	80 µl	80 µl	80 µl	80 µl
Sample	10 µl	10 µl	--	--
Standard	--	--	10 µl	--
Distilled water	10 µl	--	10 µl	20 µl
Enzyme	--	10 µl	--	--
Mix and put at room temperature for 10 minutes				
Probe	10 µl	10 µl	10 µl	10 µl
Reaction Buffer B	80 µl	80 µl	80 µl	80 µl
Dye Reagent	10 µl	10 µl	10 µl	10 µl
Mix andput in the oven at 37 °C for 5 minutes, record absorbance measured at 510 nm				

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

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

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

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

2.2. According to the weight of sample

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

2.3.According to the quantity of cells or bacteria

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

Slope: the absorbance slope of standard curve

n: the dilution factor

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

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

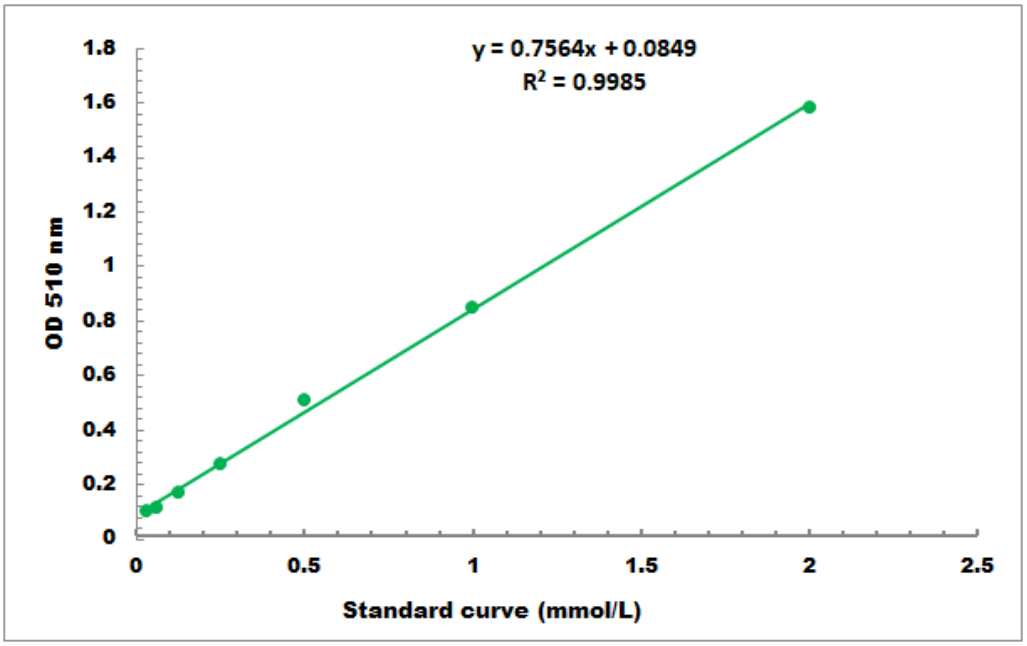
$V_{Standard}$: the volume of standard in assay procedure, mL

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

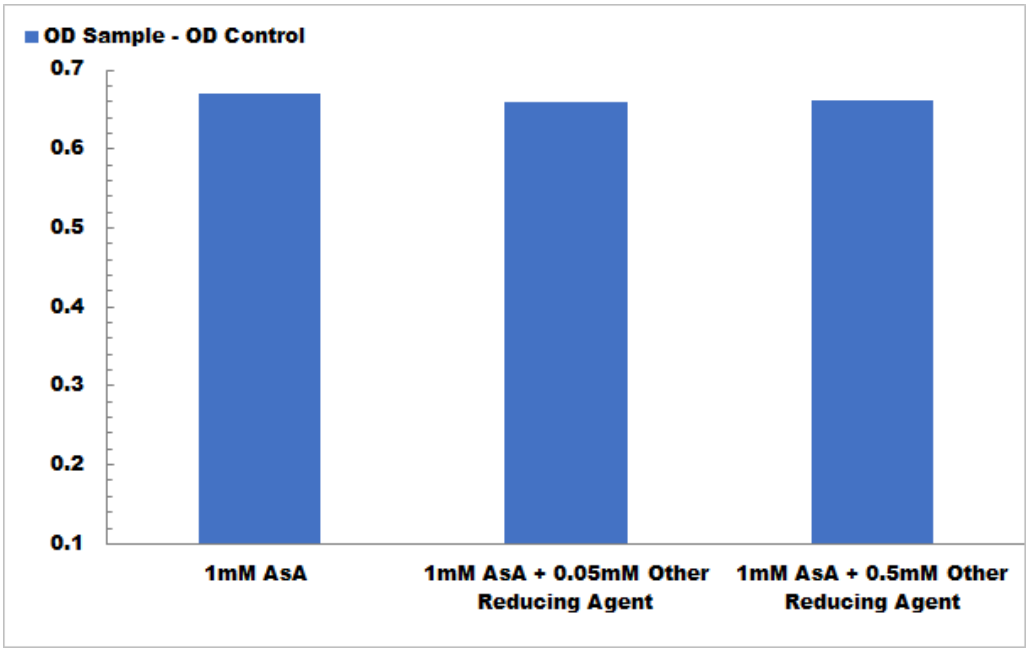
V_{Assay}: the volume of Assay Buffer in sample preparation, mL

VIII. TYPICAL DATA

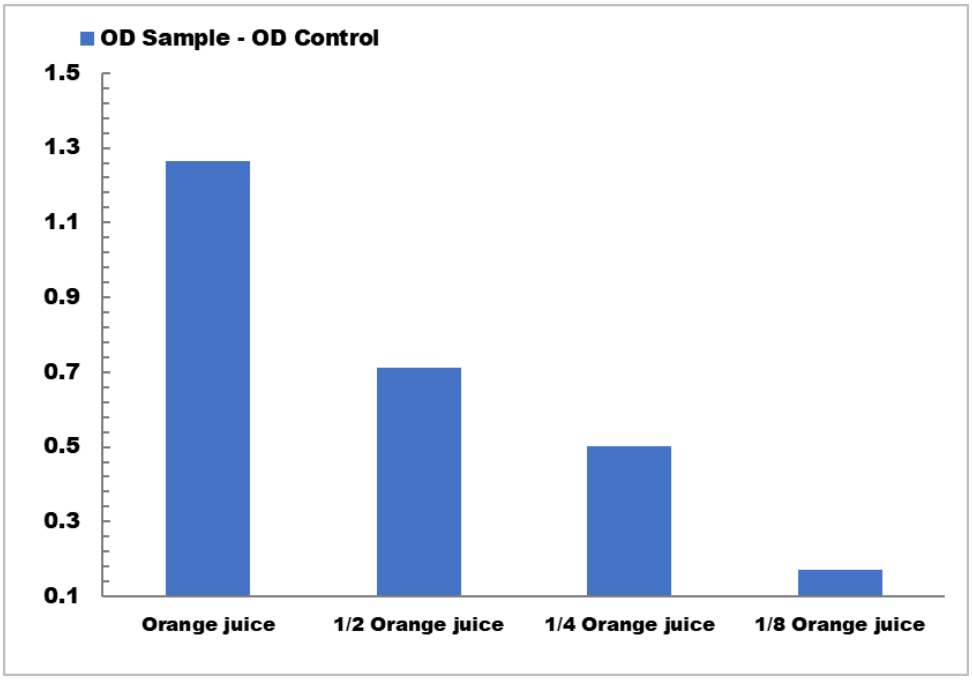
The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02 mmol/L- 2.0 mmol/L



Detection Ascorbic Acidwithother differentconcentration reducing agent



Determination of ascorbic acid in fruit juices