Ascorbic Acid Microplate Assay Kit

User Manual

Catalog # ASK1048

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

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

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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.

The reaction products can be measured at a colorimetric read out at 525 nm.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 ml x 1	4 °C
Substrate	2 ml x 1	4 °C
Dye Reagent	12 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
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Note:

Standard: add 1 ml distilled water to dissolve, mix, then add 0.01 ml into 0.99 ml distilled water, mix. The concentration of AsA is 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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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, intervation 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

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V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	40 µl	40 µl	40 µl
Substrate	20 µl	20 µl	20 µl
Mix, incubate for 5 minutes.			
Dye Reagent	120 µl	120 µl	120 µl
Mix, incubate at 37 °C for 10 minutes, record absorbance measured at 525 nm.			

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VI. CALCULATION

1. According to the protein concentration of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 1 mmol/L = 1 μmol/ml;

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

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

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

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

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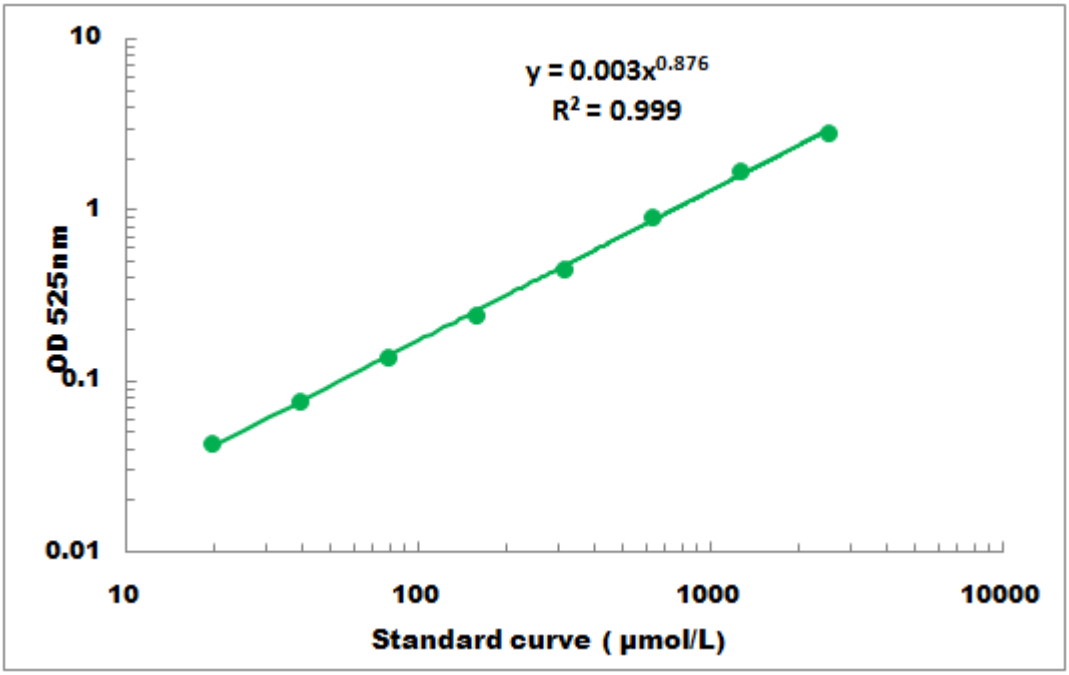
VII. TYPICAL DATA

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



Detection Range: 10 μmol/L - 3000 μmol/L

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