Ascorbic Acid Microplate Assay Kit

Catalog # AS0048

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic 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 productscan be measured at a colorimetric readout at 525 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 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
Technical Manual	1 Manual	

Note:

Standard:add 1 ml distilled water to dissolve, mix, then add 0.02 ml into 0.98 ml distilled water, mix. The concentration of AsA will be2 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

IV. 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%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents intothe 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 5minutes.			
Dye Reagent	120 µl	120 µl	120 µl
Mix,incubateat 37 °C for10 minutes, record absorbance measured at 525 nm.			

VI. CALCULATION

1. According to the protein concentration of sample

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3.According to the quantity of cells or bacteria

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

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

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 2 mmol/L = 2μmol/ml;

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

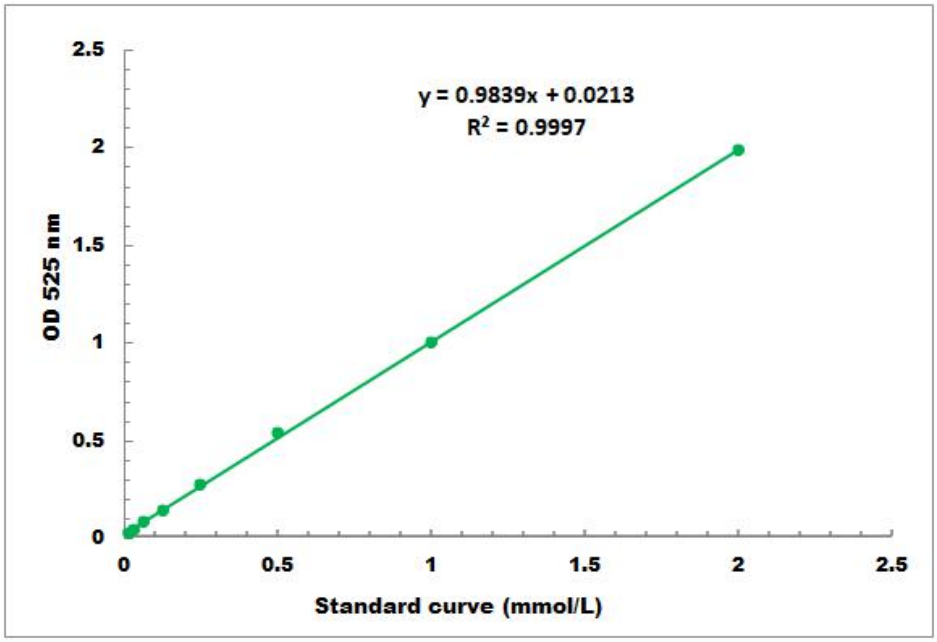
V_{Sample}: the volume of sample, 0.02 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 0.02mmol/L - 2mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES