# Ascorbic Acid Microplate Assay Kit

# User Manual

## Catalog # CAK1048

(Version 1.2C)

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.

## 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 |
| 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 be 2 mmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 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 $5 \times 10^6$ cell or bacteria, 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

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.

3. For liquid samples

Detect directly.

## 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. | | | |

**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

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

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

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 = 2 μmol/ml;

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

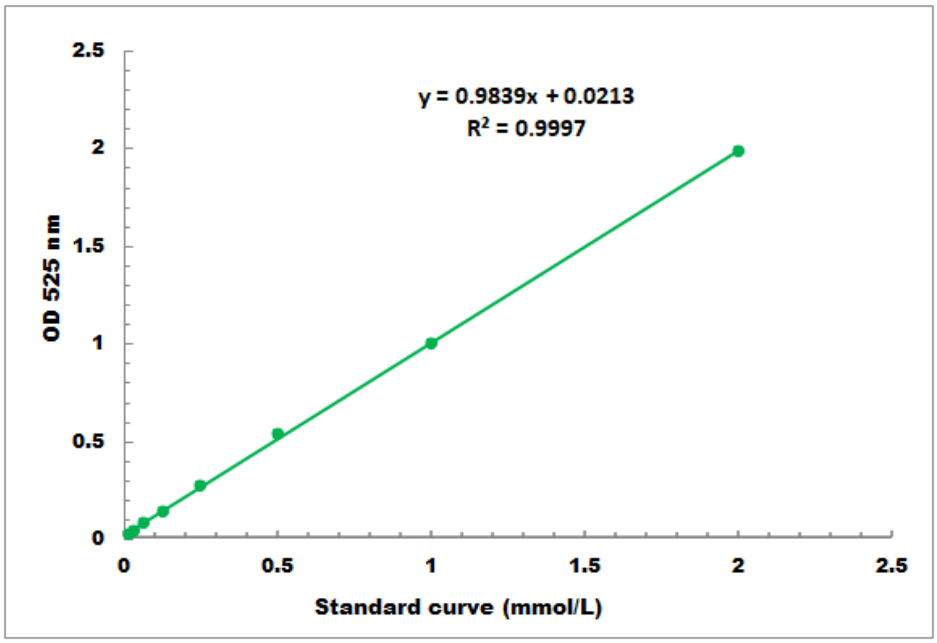
$V_{Sample}$: the volume of sample, 0.02 ml;

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

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

## VII. 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 mmol/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