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Ascorbic Acid (AsA) Content Fluorometric Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Fluorometric microplate reader

Catalog Number: AK1031

Size: 50T/24S ; 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time

Reagent

50T size

100T size

Storage

Extract solution A

Self-supplied reagent

Self-supplied reagent

-

Extract solution B

Liquid 1.25 mL×1

Liquid 2 mL×1

2-8℃

Reagent I

Liquid 10 mL×1

Liquid 15 mL×1

2-8℃

Reagent II

Liquid 5 mL×1

Liquid 8 mL×1

2-8℃

Reagent III

Powder×3

Powder×5

2-8℃

Standard

Powder×1

Powder×1

2-8℃

96-well black opaque plate

Plate×1

Plate×1

-

Sealing plate film (Cat#YA0249)

Film×2

Film×2

-

Solution Preparation:

1. Extract solution A: Self-supplied about 25mL(50T)/50mL(100T) methanol, store at room temperature. An empty white 30mL bottle is provided in the kit. Please label the reagent name by yourself.

2. Extract solution I (used for serum, plasma, cell and tissue samples): Prepare according to the sample size in the ratio of Extract solution A: distilled water: Extract solution B=900 μL: 75 μL: 25 μL (1mL, 1S) and use within the same day.

3. Extract solution II (used for standard and other samples): Prepare according to the sample size in the ratio of Extract solution A: distilled water: Extract solution B=750 μL: 225 μL: 25 μL (1mL, 1S) and use within the same day.

4. Reagent III: Add 1.5 mL of Reagent I into one bottle and dissolve it fully before use. It could be stored at 2-8℃ for 5 days or -20℃ for 10 days.

5. Standard: Add 0.568 mL distilled water to fully dissolve and prepare 100 μmol/mL standard solution before use. It could be stored at 2-8℃ for 2 weeks.

Product Description：

Ascorbic acid (AsA) is a kind of coenzyme, free radical scavenger, electron acceptor and substrate of oxalate and tartrate biosynthesis. As the most important antioxidant in plant cells, AsA plays an important role in protecting chloroplasts from oxidative damage, and is also one of the important indicators to measure the quality of crop products. Dehydroascorbic acid (DHA) is a reversible oxidation type of AsA. In vivo, it forms a redox system with ascorbic acid and has the function of electron acceptor.

AsA is oxidized to DHA by an oxidizing agent. DHA then reacts with o-phenylenediamine to form a quinoxaline derivative. Its fluorescence intensity at the excitation wavelength of 338 nm and the emission wavelength of 420 nm is proportional to the AsA content.

Reagents and Equipment Required but Not Provided：

Fluorometric microplate reader, balance, desk centrifuge, pipette, mortar/homogenizer/cell ultrasonic crusher, 96-well black opaque plate(provided), sealing plate film(provided), methanol(>98%， AR), ice and distilled water.

Procedure

I. Sample preparation:

1. Tissue: according to the proportion of tissue mass (g): Extract solution I volume (mL) of 1:5-10 (it is recommended to add 1 mL of the Extract solution I to 0.1 g of tissue). After ice bath homogenization, centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

2. Bacteria/cells: according to the number of bacteria/cells (106): the volume of the Extract solution I (mL) is 10-20:1 (it is recommended to add 1 mL of the Extract solution I to 10 million bacteria/cells), ultrasonic wave breaks bacteria/cells (ice bath, power 200W, ultrasonic 3s, interval 7s, total time 3min). Centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum (plasma): according to the proportion of serum (plasma) volume (mL): Extract solution I volume (mL) of 1:4. After incubating on ice for 10 minutes, centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

4. Other liquid samples: according to the proportion of liquid volume (mL): Extract solution II volume (mL) of 1: 10-250. After incubating on ice for 10 minutes, centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

II. Determination procedure：

1. Preheat fluorometric microplate reader for 30min, adjust excitation wavelength/emission wavelength to 338nm/420nm.

2. Standard dilution: Dilute 100 μmol/mL (100000 nmol/mL) standard solution with Extract solution II to obtain 100, 50, 25, 12.5, 6.25, 3.125nmol/mL standard solution before use.

3. Add reagents with the following list:

Reagent (μL)
Test tube
Control tube
Standard tube
Blank tube
Sample
50
50
-
-
Standard
-
-
50
-
Extract solution II
50
50
50
100
Reagent I
-
100
-
-
Reagent II
100

-
100
100
React at room temperature for 10 minutes
Reagent III
50
50
50
50
Mix well, seal the plate with a sealing film and react in the dark at room temperature for 5 minutes. Measure Ex/Em=338nm/420nm, recording as RFUT, RFUC, RFUS and RFUB respectively. Calculate ΔRFUT
= RFUT-RFUC, ΔRFUS =RFUS-RFUB. A control tube is required for each test tube. Blank tube and standard
curve only need to be measured 1-2 times.

III. Calculations：

1.
Standard curve
According to the concentration of the standard tube (x, nmol/mL) and the absorbance ΔRFUS (y, ΔRFUS), a standard curve was established. According to the standard curve, ΔRFUT (y, ΔRFUT) was brought into the formula to calculate the sample concentration (x, nmol/mL).

2.
Calculation of AsA content

1) Sample mass:
AsA content (nmol/g mass) = x×VE÷ W×F1=x÷W×F1
2) Bacteria/cells number:
AsA content (nmol/106 cell)= x×VE÷ N×F1=x÷N×F1
3) Protein concentration:
AsA content (nmol/mg prot)= x×VS÷ (VS×Cpr) ×F1=x÷Cpr×F1
4) Liquid sample volume:
AsA content (nmol/mL)= x×F2

VE: Added volume of Extract solution in the sample preparation, 1mL; Vs: Sample volume in reaction, 0.05mL; W: Sample mass, g; N: The number of bacteria/cells, count by 106; Cpr: Sample protein concentration, g/L; F1: Dilution factor of sample; F2: Dilution factor of liquid sample in the sample preparation.

Notes:

1.
If RFUT exceeds the maximum of the standard curve, it is recommended to dilute the sample with Extract solution before determination; If RFUT is below the minimum of the standard curve, it is

recommended to increase the volume of sample and decrease the volume of Extract solution II or increase the proportion of sample in the preparation before determination. The calculation formula should be multiplied by the corresponding dilution factor.

2.

Differences in the sensitivity of various fluorometric microplate readers may lead to variations in the detected RFU, which is a normal phenomenon. Provided that the reagent preparation and experimental procedure are correct and the standard curve exhibits good linearity, the detection results are considered accurate and valid.

Experimental examples:

1.

Dilute 100 μmol/mL standard solution with Extract solution II to obtain 100, 50, 25, 12.5, 6.25, 3.125nmol/mL standard solution. Measure RFU of standard tubes and establish the standard curve y = 3.8507x + 4.1898，R2=0.9967.

2.

Results of different samples:

Samples
Mass/Number
Dilution factor
RFUT
RFUC
AsA content
Unit
Broccoli
0.106g
4
262.640
155.121
1012.599 nmol/g mass
Kiwifruit
0.106g
10
315.349
87.182
5487.294 nmol/g mass
Chicken serum
-
5
119.548
97.604
23.053 nmol/mL

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Orange juice
-
160
159.429
104.042
2127.289 nmol/mL
3T3 cells
10×106
1
126.411 115.269 0.090 nmol/106 cell