

Ascorbic Acid and Total Ascorbic Acid Content Assay Kit (Colorimetric Method)

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC4635

Size: 100T/48S

Components:

Extracting solution: Liquid 70 mL×1. Storage at 4°C.

Reagent I: Powder ×1. Storage at -20°C. Before use, add 2 mL of distilled water to each tube and dissolve it completely. The unused reagents is divided and then stored at -20°C.

Reagent II: Liquid 10 mL ×1. Storage at 4°C.

Reagent III: Liquid 2 mL ×1. Storage at 4°C.

Reagent IV: Liquid 20 mL×1. Storage at 4°C.

Reagent V: Liquid 15 mL×2. Storage at 4°C.

Reagent VI: Powder ×1. Storage at 4°C. Before use, add 10 mL of 70% ethanol solution to Reagent IV and dissolve it completely. The unused reagents is divided and then stored at -20°C.

Reagent VII: Liquid 10 mL ×1. Storage at 4°C.

Standard solution: Powder ×1, Storage at 4°C. Before use, add 1.136 mL of the Extracting solution to fully dissolve; absorb 0.02 mL of the above solution, add 0.98 mL of the Extracting solution, and mix well, that is, 1000 nmol/mL AsA standard solution.

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 reductive and can reduce Fe^{3+} to Fe^{2+} . Fe^{2+} and 2,2'-bipyridine form a pink complex with a characteristic absorption peak at 525 nm. DTT can reduce DHA to produce AsA, which can be used to detect the content of total ascorbic acid (AsA+DHA) in samples.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, low temperature centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ethanol, ice and distilled water.

Procedure:

I. Sample preparation (the sample size can be adjusted appropriately, and the specific proportion can be referred to the literature):

1. Preparation of tissue samples: according to the ratio of mass (g): the volume of Extracting

solution (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extracting solution), add Extracting solution, homogenize in ice bath, centrifuge at 4°C, 13000g for 10 min, take supernatant for testing.

2. Preparation of bacteria and cell samples: according to the cell number (10^4): the volume of Extracting solution (mL) is 500 ~ 1000:1 (it is recommended to add 1 mL Extracting solution to 5 million cells), ice bath ultrasonic wave is used to crush cells (power 300W, ultrasonic 3 seconds, interval 7 seconds, total time 3 min); centrifugation at 4°C, 13000 g for 10 min, take supernatant for testing.

3. Serum (plasma): take 500 μ L of sample and add 500 μ L of Extracting solution, vortex mixing, centrifuge at 4°C, 13000g for 10 min, and then take the supernatant for testing.

II. Determination procedure:

1. Preheat the Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 525 nm, set zero with distilled water.

2. Add reagents with the following list:

Reagent (μ L)	Test tube (T)	Control tube(C)	Blank tube1(B1)	Blank tube2(B2)	Standard tube(S)
Sample	15	15	-	-	-
Extracting solution	-	-	15	15	-
Standard solution	-	-	-	-	15
Reagent II	60	60	60	60	60
Reagent IV	75	75	75	75	75
Reagent V	60	60	60	60	60
Reagent VI	60	-	60	-	60
70% ethanol solution	-	60	-	60	-
Reagent VII	30	30	30	30	30

Mix well, react with water bath at 42°C accurately for 40 minutes, cool it in cold water, take 200 μ L of reaction solution and measure the absorbance value at 525 nm. record them as A_T , A_C , A_{B1} , A_{B2} and A_S respectively. Calculate $\Delta A_{T1} = (A_T - A_C) - (A_{B1} - A_{B2})$, $\Delta A_{S1} = A_S - A_{B1}$.

Note: when adding Reagent VII, put the gun head below the liquid level and do not drop it in the air, otherwise the liquid will be turbid. Blank tube 1, Blank tube 2 and Standard tube only need to be measured 1-2 times.

3. Determination of T-AsA content

According to the operation table, add the following reagents:

Reagent (μ L)	Test tube (T)	Control tube (C)	Blank tube1 (B1)	Blank tube2 (B2)	Standard tube (S)

Sample	15	15	-	-	-
Extracting solution	-	-	15	15	-
Standard solution	-	-			15
Reagent I	15	15	15	15	15
Reagent II	30	30	30	30	30
Mix well and react in water bath at 42°C for 15 min.					
Reagent III	15	15	15	15	15
Mix well and let stand at room temperature for 1 min.					
Reagent IV	75	75	75	75	75
Reagent V	60	60	60	60	60
Reagent VI	60	-	60	-	60
70% ethanol solution	-	60	-	60	-
Reagent VII	30	30	30	30	30
Mix well, react with water bath at 42°C accurately for 40 minutes, cool it in cold water, take 200 μL of reaction solution and measure the absorbance value at 525 nm. record them as A_T , A_C , A_{B1} , A_{B2} and A_S respectively. Calculate $\Delta A_{T2} = (A_T - A_C) - (A_{B1} - A_{B2})$, $\Delta A_{S2} = A_S - A_{B1}$.					

Note: when adding Reagent VII, put the gun head below the liquid level and do not drop it in the air, otherwise the liquid will be turbid. Blank tube 1, Blank tube 2 and Standard tube only need to be measured 1-2 times.

III. Calculation:

A、 Calculation formula of AsA content

(1) Calculated by sample quality

$$\text{AsA content (nmol/g mass)} = (C_S \times \Delta A_{T1} \div \Delta A_{S1} \times V_S) \div (W \times V_S \div V_{ST}) = 1000 \times \Delta A_{T1} \div \Delta A_{S1} \div W$$

(2) Calculated by the number of bacteria or cells

$$\text{AsA content } (\mu\text{g}/10^4 \text{ cells}) = (C_S \times \Delta A_{T1} \div \Delta A_{S1} \times V_S) \div (\text{cell number} \times V_S \div V_{ST}) = 1000 \times \Delta A_{T1} \div \Delta A_{S1} \div \text{cell number}$$

(3) Calculated according to the volume of liquid

$$\text{AsA content } (\mu\text{g}/\text{mL}) = C_S \times \Delta A_{T1} \div \Delta A_{S1} \times 2 = 2000 \times \Delta A_{T1} \div \Delta A_{S1}$$

C_S : standard tube concentration, 1000 nmol/mL; V_{ST} : volume of supernatant after centrifugation, 1.0 mL. V_S : volume of supernatant during extraction, 0.015 mL; W : sample mass, g; 2: Dilution ratio, (500 μL of liquid + 500 μL of Extracting solution)/500 μL of liquid = 2.

B、 Calculation formula of T-AsA content

(1) Calculated by sample quality

$$\text{AsA content (nmol/g mass)} = (C_S \times \Delta A_{T2} \div \Delta A_{S2} \times V_S) \div (W \times V_S \div V_{ST}) = 1000 \times \Delta A_{T2} \div \Delta A_{S2} \div W$$

(2) Calculated by the number of bacteria or cells

$$\text{AsA content } (\mu\text{g}/10^4 \text{ cells}) = (C_S \times \Delta A_{T2} \div \Delta A_{S2} \times V_S) \div (\text{cell number} \times V_S \div V_{ST}) = 1000 \times \Delta A_{T2} \div \Delta A_{S2} \div \text{cell}$$

number

(3) Calculated according to the volume of liquid

$$\text{AsA content } (\mu\text{g/mL}) = C_S \times \Delta A_{T2} \div \Delta A_{S2} \times 2 = 2000 \times \Delta A_{T2} \div \Delta A_{S2}$$

C_S : standard tube concentration, 1000 nmol/mL; V_{ST} : volume of supernatant after centrifugation, 1.0 mL. V_S : volume of supernatant during extraction, 0.015 mL; W : sample mass, g; 2: Dilution ratio, (500 μ L of liquid + 500 μ L of Extracting solution)/500 μ L of liquid = 2.

C、 Calculation formula of DHA content

$$\text{DHA content} = \text{T-AsA content} - \text{AsA content}$$

(1) Calculated by sample quality

$$\text{AsA content (nmol/g mass)} = 1000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1}) \div W$$

(2) Calculated by the number of bacteria or cells

$$\text{AsA content } (\mu\text{g}/10^4 \text{ cells}) = 1000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1}) \div \text{cell number}$$

(3) Calculated according to the volume of liquid

$$\text{AsA content } (\mu\text{g/mL}) = 2000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1})$$

Note:

1. When adding Reagent VII, put the gun head below the liquid level and do not drop it in the air, otherwise the liquid will be turbid.
2. Blank tube 1, Blank tube 2 and Standard tube only need to be measured 1-2 times.
3. When a is greater than 1.5, it is recommended to dilute the sample with the Extracting solution before determination, and multiply it by the corresponding dilution multiple during calculation.
4. The kit can be used to detect the content of AsA or T-AsA in samples alone, or calculate the content of DHA after detecting the content of AsA and T-AsA at the same time.
5. The samples are detected on the same day after extraction.

Related products:

BC1230/BC1235 Ascorbic Acid (AsA) Assay Kit

BC1240/BC1245 Dehydroascorbic Acid (DHA) Assay Kit

BC1260/BC1265 Ascorbic Acid Oxidase (AAO) Activity Assay Kit

BC0220/BC0225 Ascorbate Peroxidase (APX) Activity Assay Kit

BC0650/BC0655 Single Dehydroascorbate Reductase (MDHAR) Activity Assay Kit

BC0660/BC0665 Dehydroascorbate Reductase (DHAR) Activity Assay Kit