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

Cat No: BC4630

Size: 50T/24S

Components:

Extracting solution：Liquid 40 mL×1. Storage at 4℃.

Reagent I: Powder ×1. Storage at -20℃. Before use, add 3.3 mL of distilled water to each tube and dissolve it completely. The unused reagents is divided and then stored at -20℃.

Reagent II: Liquid 20 mL ×1. Storage at 4℃.

Reagent III: Liquid 3 mL ×1. Storage at 4℃.

Reagent IV: Liquid 30 mL×1. Storage at 4℃.

Reagent V: Liquid 20 mL×1. Storage at 4℃.

Reagent VI: Powder ×1. Storage at 4℃. Before use, add 12 mL of 70% ethanol solution to Reagent IV and dissolve it completely. The unused reagents is divided and then stored at -20℃.

Reagent VII: Liquid 12 mL ×1. Storage at 4℃.

Standard solution: Powder ×1, Storage at 4℃. Before use, add 1.136 mL of the Extracting solution to fully dissolve; absorb 0.01 mL of the above solution, add 0.99 mL of the Extracting solution, and mix well, that is, 500 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 an 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 tempareture centrifuge, transferpettor, 1 mL 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℃, 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℃, 13000 g for 10 min, take supernatant for testing.

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

II. Determination procedure:

1. Preheat the Spectrophotometer for 30 minutes, adjust the wavelength to 525 nm, set zero with distilled water.
2. Add reagents with the following list:

Reagent（μL）	Test tube (T)	Control tube(C)	Blank tube1(B1)	Blank tube2(B2)	Standard tube(S)
Sample	50	50	-	-	-
Extracting solution	-	-	50	50	-
Standard solution	-	-	-	-	50
Reagent II	200	200	200	200	200
Reagent IV	250	250	250	250	250
Reagent V	200	200	200	200	200
Reagent VI	200	-	200	-	200
70% ethanol solution	-	200	-	200	-
Reagent VII	100	100	100	100	100
Mix well, react with water bath at 42℃ accurately for 40 minutes, cool it in cold water, 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（μL）	Test tube (T)	Control tube (C)	Blank tube1 (B1)	Blank tube2 (B2)	Standard tube (S)

Sample	50	50	-		-
Extracting solution	-	-	50	50	-
Standard solution	-	-			50
Reagent I	50	50	50	50	50
Reagent II	100	100	100	100	100
Mix well and react in water bath at 42℃ for 15 min.					
Reagent III	50	50	50	50	50
Mix well and let stand at room temperature for 1 min.					
Reagent IV	250	250	250	250	250
Reagent V	200	200	200	200	200
Reagent VI	200	-	200	-	200
70% ethanol solution	-	200	-	200	-
Reagent VII	100	100	100	100	100
Mix well, react with water bath at 42℃ accurately for 40 minutes, cool it in cold water, 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

AsA content (nmol/g mass)= $(C_S\times\Delta A_{T1}\div\Delta A_{S1}\times V_S)\div(W\times V_S\div V_{ST})=500\times\Delta A_{T1}\div\Delta A_{S1}\div W$

(2) Calculated by the number of bacteria or cells

AsA content (μg/10^4 cells)= $(C_S\times\Delta A_{T1}\div\Delta A_{S1}\times V_S)\div(\text{cell number}\times V_S\div V_{ST})=500\times\Delta A_{T1}\div\Delta A_{S1}\div$cell number

(3) Calculated according to the volume of liquid

AsA content (μg/mL)= $C_S\times\Delta A_{T1}\div\Delta A_{S1}\times2=1000\times\Delta A_{T1}\div\Delta A_{S1}$

C_S: standard tube concentration, 500 nmol/mL; V_{ST}: volume of supernatant after centrifugation, 1.0 mL. V_S: volume of supernatant during extraction, 0.05 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

AsA content (nmol/g mass)= $(C_S\times\Delta A_{T2}\div\Delta A_{S2}\times V_S)\div(W\times V_S\div V_{ST})=500\times\Delta A_{T2}\div\Delta A_{S2}\div W$

(2) Calculated by the number of bacteria or cells

AsA content (μg/10^4 cells)= $(C_S\times\Delta A_{T2}\div\Delta A_{S2}\times V_S)\div(\text{cell number}\times V_S\div V_{ST})=500\times\Delta A_{T2}\div\Delta A_{S2}\div$cell

number

(3) Calculated according to the volume of liquid

AsA content (μg/mL)= $C_S \times \Delta A_{T2} \div \Delta A_{S2} \times 2 = 1000 \times \Delta A_{T2} \div \Delta A_{S2}$

C_S: standard tube concentration, 500 nmol/mL; V_{ST}: volume of supernatant after centrifugation, 1.0 mL. V_S: volume of supernatant during extraction, 0.05 mL; W: sample mass, g; 2: Dilution ratio, (500 μL of liquid + 500 μL of Extracting solution)/500 μL of liquid = 2.

C、Calculation formula of DHA content

DHA content =T-AsA content -AsA content

(1) Calculated by sample quality

AsA content (nmol/g mass)= $500 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1}) \div W$

(2) Calculated by the number of bacteria or cells

AsA content (μg/10^4 cells)= $500 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1}) \div$cell number

(3) Calculated according to the volume of liquid

AsA content (μg/mL) =$1000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1})$

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, 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.

Experimental example:

1. Take 0.1g of winter jujube for sample processing, and operate according to the determination steps. The results are as follows: $\Delta A_{T1} = (A_T - A_C) - (A_{B1} - A_{B2}) = (0.44-0.019)-(0.025-0.009) = 0.405$, $\Delta A_{S1} = A_S - A_{B1} = 0.341-0.018 = 0.323$, $\Delta A_{T2} = (A_T - A_C) - (A_{B1} - A_{B2}) = (0.591-0.020) - (0.024-0.008) = 0.555$, $\Delta A_{S2} = A_S - A_{B1} = 0.375-0.017 = 0.358$.

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