Ascorbic Acid (AsA) and Total Ascorbic Acid (T-AsA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/microplate reader

Cat No: BC4635

Size: 100T/48S

Components:

Extract Solution: 70 mL×1, storage at 4℃.

Reagent I: Powder×1, store at -20℃. Add 2 mL of distilled water when the solution will be used. The unused reagents can be stored at -20℃ and aviod light.

Reagent II: Liquid 10 mL×1, store at 4℃.

Reagent III: Liquid 2 mL×1, store at 4℃ and aviod light.

Reagent IV: Liquid 20 mL×1, store at 4℃.

Reagent V: Liquid 15 mL×1, store at 4℃.

Reagent VI: Powder×1, store at 4℃. Add 10 mL of 70% ethanol(v/v) before use, mix thoroughly.

Reagent VII: Liquid 10 mL×1, store at 4℃.

Standard: Powder×1, store at 4℃ and aviod light. Add 1.136 mL of extract solution before use, mix thoroughly. Add 0.98 mL distilled water to 0.02 mL of above standard solution, mix thoroughly and to be prepared as 1000 nmol/mL AsA standard solution for use.

Description:

AsA is also called Vitamin C. AsA is the substrate of coenzyme, free radical scavenger, electron copolymer/receptor, biosynthesis of oxalate and tartrate. As the most important antioxidant in plant cells, AsA has important function in protecting chloroplast from oxidizing. It is also one of the important indexes to measure the quality of crop products. DHA is a reversible oxidized form of AsA. It forms a redox system with ascorbic acid in the living body and has the function of an electron acceptor.

ASA has reductive ability, which 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 generate ASA, for this can be used to detect the total ascorbic acid(ASA + DHA) content of samples.

Required but not provided

Mortar, ice, low temperature centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well plate, transferpettor, ethanol and distilled water.

Protocol:

I. Sample extraction:

1. Tissue:

Accordance the ratio of tissue(g) : extract solution volume (mL)=1: 5~10, (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 13000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Bacteria or cells:

Accordance the ratio of cells amount(10^4) : extract solution volume (mL)=500~1000: 1, (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 300w, ultrosonic 3s, interval 7s for 3 minutes). Centrifuge at 13000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

3. Serum:

Add 500 μL of extract solution to 500 μL of sample, vortex mixing. Centrifuge at 13000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

II. Determination procedure

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

2. Determination of ASA content:

Add reagents with the following list:

Reagent (μL)	Test tube (T)	Contrast tube (C)	Blank tube 1 (B1)	Blank tube 2 (B2)	Standard tube (S)
Sample	15	15	-		-
Extract 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	-	60	-	60	-
Reagent VII	30	30	30	30	30
Mix well, react in water bath at 42℃ for 40 minutes, cooling with cold water. Take 200 μL in a micro glass cuvette/96 well plate to determine the absorption value at 525 nm, record as A_T, A_C, A_{B1}, A_{B2} and A_S. 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 tips of transferpettor under the liquid level, do not drop it in the air, otherwise the liquid will be turbid. The Blank tube 1, Blank tube 2 and Standard tube only needs to be measured one or twice.

3. Determination of ASA content:

Add reagents with the following list:

Reagent (μL)	Test tube (T)	Contrast tube (C)	Blank tube 1 (B1)	Blank tube 2 (B2)	Standard tube (S)
Sample	15	15	-	-	-

Extract solution	-	-	15	15	-
Standard solution	-	-	-	-	15
Reagent I	15	15	15	15	15
Reagent II	30	30	30	30	30
Mix well, react in water bath at 42℃ for 15 minutes.					
Reagent III	15	15	15	15	15
Mix well, place at room temperature for 1 minute.					
Reagent IV	75	75	75	75	75
Reagent V	60	60	60	60	60
Reagent VI	60	-	60	-	60
70% ethanol	-	60	-	60	-
Reagent VII	30	30	30	30	30
Mix well, react in water bath at 42℃ for 40 minutes, cooling with cold water. Take 200 μL in a micro glass cuvette/96 well plate to determine the absorption value at 525 nm, record as A_T, A_C, A_{B1}, A_{B2} and A_S. 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 tips of transferpettor under the liquid level, do not drop it in the air, otherwise the liquid will be turbid. The Blank tube 1, Blank tube 2 and Standard tube only needs to be measured one or twice.

III. Calculation of ASA/T-ASA Content:

A. Calculation of ASA content:

1. Sample weight:

$AsA(nmol/g) =[C_S\times\Delta A_{T1}\div\Delta A_{S1}\times V_{SV}]\div(W\times V_{SV}\div V_{STV}) =1000\times\Delta A_{T1}\div\Delta A_{S1}\div W$

2. Cells:

$AsA(nmol/10^4\ cell) =[C_S\times\Delta A_{T1}\div\Delta A_{S1}\times V_{SV}]\div(N\times V_{SV}\div V_{STV})=1000\times\Delta A_{T1}\div\Delta A_{S1}\div N$

3. Liquids:

$AsA\ (nmol/mL) =[C_S\times\Delta A_{T1}\div\Delta A_{S1}\times V_{SV}]\times2=2000\times\Delta A_{T1}\div\Delta A_{S1}$

C_S: 1000 nmol/mL;

V_{STV}: The volume of supernatant after centrifugation, 1 mL;

V_{SV}: The volume of supernatant added into the reaction system , 0.0125 mL;

W: Sample weight, g;

V_{Liq}: The volume of sample added during extraction , 0.5 mL;

V_E: The volume of extract solution added during extraction , 0.5 mL;

2: The ratio of dilution, $(V_{Liq}+ V_E)/V_{Liq}$ = (500 μL+500 μL)/500 μL = 2.

N: The number of cells.

B. Calculation of T-ASA content:

$T\text{-}AsA(nmol/g) =[C_S\times\Delta A_{T2}\div\Delta A_{S2}\times V_{SV}]\div(W\times V_{SV}\div V_{STV}) =1000\times\Delta A_{T2}\div\Delta A_{S2}\div W$

2. Cells:

$T\text{-}AsA(nmol/10^4\ cell) =[C_S\times\Delta A_{T2}\div\Delta A_{S2}\times V_{SV}]\div(N\times V_{SV}\div V_{STV})=1000\times\Delta A_{T2}\div\Delta A_{S2}\div N$

3. Liquids

T-AsA (nmol/mL) =$[C_S \times \Delta A_{T2} \div \Delta A_{S2} \times V_{SV}] \times 2 = 2000 \times \Delta A_{T2} \div \Delta A_{S2}$

C_S: 1000 nmol/mL;

V_{STV}: The volume of supernatant after centrifugation, 1 mL;

V_{SV}: The volume of supernatant added into the reaction system , 0.0125 mL;

W: Sample weight, g;

V_{Liq}: The volume of sample added during extraction , 0.5 mL;

V_E: The volume of extract solution added during extraction , 0.5 mL;

2: The ratio of dilution, $(V_{Liq} + V_E)/V_{Liq}$ = (500 μL+500 μL)/500 μL = 2.

N: The number of cells.

C. Calculation of DHA content:

1. Sample weight:

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

2. Cells:

DHA (nmol/10^4 cell) =$1000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1}) \div N$

3. Liquids:

DHA (nmol/mL) =$2000 \times (\Delta A_{T2} \div \Delta A_{S2} - \Delta A_{T1} \div \Delta A_{S1})$

W: Sample weight, g;

N: The number of cells.

Note:

1. When adding Reagent VII, put the tips of transferpettor under the liquid level, do not drop it in the air, otherwise the liquid will be turbid.
2. The Standard tube only needs to be measured one or twice.
3. When the measured absorption value is greater than 1.5, it is recommended to measure after dilution with extract solution, multiply the corresponding dilution ratio in calculation.
4. This kit can be used to detect ASA or T-ASA content in samples alone, or calculate DHA content after simultaneous detection of ASA and T-ASA content.
5. The samples need tested on the same day after extraction.

Experimental Examples:

1. Take 0.1g of haw for sample processing, follow the measurement steps to calculate ΔA1t = (At-Ac)-(Ab1-Ab2) =（0.178-0.063）-（0.058-0.048）=0.105, ΔA1s=As-Ab1=0.386-0.058=0.328, ΔA2t=(At-Ac)-(Ab1-Ab2)=（0.308-0.066）-（0.057-0.047）=0.232, ΔA2s=As-Ab1=0.466-0.057=0.409, calculate the AsA content and T-AsA content according to the sample weight, and get:

AsA (nmol/g weight)=$1000 \times \Delta A1t \div \Delta A1s \div W$=3201.2 nmol/g weight

T-AsA (nmol/g weight) = $1000 \times \Delta A2t \div \Delta A2s \div W$= 5672.4 nmol/g weight

Related Products:

BC1230/BC1235 Ascorbic Acid(AsA) Content Assay Kit

BC1240/BC1245 Dehydroascorbic Acid(DHA) Content Assay Kit

BC1260/BC1265 Ascorbic Acid Oxidase(AAO) Activity Assay Kit
BC0220/BC0225 Ascorbate Peroxidase(APX) Activity Assay Kit
BC0650/BC0655 Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit
BC0660/BC0665 Dehydroascorbate Reductase(DHAR) Activity Assay Kit