

## Reduced Vitamin C (VC) Content Assay Kit (DCPIP, Titration Method)

Cat. No.: R1515897 | Pack size: 50T | Storage: Store at 2-8°C, Protected from light, Room temperature

### Product Information

Item	Description
Specifications & Purity	BioReagent
Application	Cell Metabolism
Sample type	Fruits
Method Of Detection	Titration Method
Detected Species Object	plant
Storage Conditions	Store at 2-8°C, Protected from light, Room temperature
Shipped In	Wet ice
Stability And Storage	Each component has a shelf life of 12 months under corresponding storage conditions. R1515897A: Store at room temperature long term (12 months). Store in the dark. R1515897B/D: Store at room temperature long term (12 months). R1515897C: Store at 2-8°C long term (12 months). Store in the dark.

### Product Description

Vitamin C is an organic compound with antioxidant properties, also known as ascorbic acid. It is an essential nutrient for higher primates and a small number of other organisms. In organisms, vitamin C acts as both an antioxidant and a coenzyme. It is an acidic hexose derivative and an enol hexonolactone, protecting the body from free radical damage. It is widely abundant in fresh vegetables and fruits. There are L-ascorbic acid and D-ascorbic acid, both possessing strong

reducibility, yet only L-ascorbic acid exhibits biological activity in humans. L-ascorbic acid exists in reduced and oxidized forms. The reduced form accounts for over 90% in ordinary vegetables and fruits. Reduced L-ascorbic acid is easily oxidized into L-dehydroascorbic acid, which can also be reduced back to reduced L-ascorbic acid, commonly referred to as oxidized ascorbic acid.

The detection principle of this kit: Reduced vitamin C is readily oxidized to oxidized vitamin C by oxidized 2,6-dichlorophenolindophenol (DCPIP). DCPIP serves as both an oxidant and an acid-base indicator. It appears blue in neutral and alkaline solutions, red in acidic solutions, and colorless when reduced. When titrating acidic vitamin C solution with DCPIP, the dropped DCPIP is immediately reduced and decolorized before vitamin C is fully oxidized. Once vitamin C is completely oxidized, excess DCPIP turns pink. The titration endpoint is reached when the solution changes from colorless to pale red. Without impurity interference, the amount of standard DCPIP reduced by the sample is proportional to the reduced vitamin C content in the sample. This kit is widely applied to determine reduced vitamin C in vegetables, fruits and their products. This kit is for scientific research only, not for clinical diagnosis or other applications.

## Self-Prepared Materials

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1. Tissue homogenizer, blender, filter paper, funnel, 50 mL volumetric flask.
2. 100 mL Erlenmeyer flask, 10 mL pipette, 5 mL micro burette.

## Procedure (For Reference Only)

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1. Prepare 1× tissue homogenate: Dilute 12× tissue homogenate with distilled water at a ratio of 1:11.
2. Prepare sample extract: Wash fresh vegetables/fruits thoroughly, blot surface moisture or air-dry naturally. Weigh 5 g sample, add 5 mL 1× tissue homogenate, homogenize into slurry. Filter with filter paper, transfer filtrate into a 50 mL volumetric flask. Re-homogenize residue 1–2 times with 1× homogenate, combine all filtrates and dilute to a total volume of 50 mL.
3. Prepare vitamin C standard solution: Add a small amount of 1× tissue homogenate to 10 mg vitamin C standard, mix well, then transfer completely into homogenate to obtain 0.1 mg/mL vitamin C standard solution.

*Note: Complete and accurate dissolution is required to avoid inaccurate standard concentration. Store prepared solution at 4 °C away from light, valid for 4 weeks; freshly prepared solution is recommended.*

4. Prepare DCPIP standard solution: Add a small amount of DCPIP diluent to 50 mg DCPIP powder, mix uniformly, transfer fully into diluent and fix volume to 250 mL to obtain 0.2 mg/mL DCPIP standard solution.

**Note:** Ensure full dissolution. Store at 4°C protected from light, valid for 1 week.

5. Calibrate DCPIP standard solution: Accurately pipette 1 mL 0.1 mg/mL vitamin C standard into an Erlenmeyer flask, add 9 mL 1× tissue homogenate. Titrate with DCPIP solution until pale red color persists for 15 seconds, record dye consumption ( $V_1$ ).

Take another flask with 10 mL 1× homogenate as blank control, titrate identically and record blank consumption ( $V_2$ ). Calculate titration factor T (mg vitamin C per mL DCPIP). Repeat 3 times and take average value.

6. Sample titration: Pipette 10 mL sample filtrate into a 100 mL Erlenmeyer flask, titrate following the same steps and record dye consumption ( $V_3$ ). Repeat 3 times for average result.

## Calculation

Vitamin C content in 100 g sample (mg) =  $(V_3 - V_2) \times V_t / (V_s \times m) \times T \times 100$

$T$  (mg/mL) =  $0.1 \times 1 / (V_1 - V_2)$

Where:  $V_t$  = Total volume of sample extract (mL)  $V_s$  = Volume of sample extract used for titration (mL).

$V_1$  = DCPIP volume for standard calibration (mL).

$V_2$  = DCPIP volume for blank titration (mL).

$V_3$  = DCPIP volume for sample titration (mL).

T = Titration titer, mass of vitamin C equivalent to 1 mL DCPIP (mg/mL).

m = Mass of raw sample used for extraction (g).

## Contents & Storage

Item No.	Appearance	Components	50T	Storage
R1515897A	Solid	Vitamin C Standard	10 mg	RT. Store in the dark.
R1515897B	Liquid	Tissue Homogenate Buffer (12×)	250 ml	RT.
R1515897C	Solid	2,6- Dichlorophenolin dophenol (DCPIP)	50 mg	2-8. Store in the dark.
R1515897D	Liquid	DCPIP Diluent	250 ml	RT.

## Precautions

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1. 12× tissue homogenate may develop milky turbidity after long storage or low-temperature preservation. Slight turbidity does not affect detection and can be used directly; discard severely turbid reagent.
2. Store prepared vitamin C standard at -20°C away from light, valid for 4 weeks. Fresh preparation is preferred.
3. Store DCPIP standard at 4 protected from light, valid for 1 week. To save reagent, weigh 10 mg DCPIP, dilute to 50 mL with special diluent for use.
4. The supplied tissue homogenate is suitable for most vegetable and fruit samples and inhibits ascorbate oxidase activity.
5. Pine needle samples shall be extracted with 1% hydrochloric acid solution.
6. Samples rich in Fe<sup>2+</sup> shall be extracted with 8% acetic acid, as Fe<sup>2+</sup> reacts slowly with DCPIP dye.
7. Untested samples can be stored at 2–8°C in dark and remain stable within 3 days.
8. Avoid contact with iron and copper utensils during sample cutting, and operate quickly.
9. Add a few drops of butanol or octanol to reduce excessive foam from fruit and vegetable pulp.
10. Discard initial several milliliters of filtrate when using dry filter paper, or collect supernatant after centrifugation.
11. Centrifuge at low speed and use supernatant for titration if filtration is difficult.
12. Keep sample extract away from direct sunlight to prevent accelerated oxidation of ascorbic acid.
13. For highly pigmented extracts, decolorize with kaolin before titration, or add 1 mL chloroform. The chloroform layer turns pale red at endpoint. Xylene extraction colorimetry is also applicable.
14. Liquid samples such as orange juice do not require grinding. Mix 5 mL juice with equal volume of 1× homogenate directly for titration.
15. Complete titration within 2 minutes to prevent oxidation of reduced vitamin C.
16. DCPIP consumption is recommended between 1–4 mL, optimally around 2 mL.
17. Add DCPIP rapidly at first, then dropwise with continuous shaking until stable pale red lasts 15 s, to minimize impurity interference.
18. Dilute high-concentration samples with distilled water before retesting, and multiply final result by dilution factor.
19. Wear lab coat and disposable gloves for safe operation.
20. Use opened reagents promptly to guarantee experimental accuracy.

## Appendix

All reagents in this kit are accurately weighed or volumetrically fixed. If vitamin C standard solution deviates after long storage, recalibrate its concentration as follows:

Dilute 0.1 mg/mL vitamin C standard to 0.02 mg/mL with 1× homogenate. Pipette 5 mL diluted standard into flask, add 0.5 mL 6% potassium iodide solution and 3 drops 1% starch indicator. Titrate with 0.001 mol/L potassium iodate standard solution until stable blue color appears.

Vitamin C concentration (mg/mL) =  $0.088 \times V_1 \div V_2$

Where:  $V_1$  = Consumed potassium iodate volume (mL).

$V_2$  = Volume of vitamin C standard used (mL).

0.088 = Vitamin C equivalent mass of 1 mL potassium iodate solution (mg).

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