COSMO BIO CO., LTD.
Inspiration for Life Science

Vitamin C Assay Kit

(DNPH method)

Product No. SML-ROIK02-EX

Vitamin C (L-Ascorbic acid) is water-soluble vitamin with strong reducing action and is an important coenzyme for internal hydroxylation reactions (e.g. collagen). Vitamin C is found in both reduced form (ascorbic acid (AsA)) and oxidized form (dehydroascorbic acid (DHAsA)). This Vitamin C Assay Kit measures total vitamin C (AsA + DHAsA).

The method of this kit is a refinement of the colorimetric assay described by Daniel (1973).

【 REAGENTS 】 [Sufficient for 100 assay points]※

1) Reagent① : 1 vial (2 ml)
   Oxidizing agent
2) Reagent② : 1 vial (10 ml)
   5% Metaphosphoric acid/2% $SnCl_2$
3) Reagent③ : 1 vial (Dissolve in 3 ml 44% sulfuric acid)
   2,4-Dinitrophenylhydrazine (DNPH)
4) Reagent④ : 1 vial (10 ml)
   5% Metaphosphoric acid
5) Vitamin C Standard Stock Solution : 1 vial (1 ml)

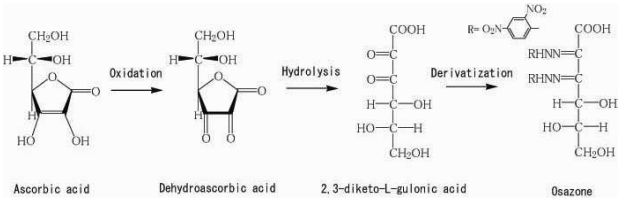
【 PREPARATION OF REGENT 】

Note: 44% Sulfuric Acid is used to prepare reagent③ and 85% Sulfuric acid is added during the assay protocol. Please prepare appropriate dilutions from sulfuric acid stock solution. CAUTION: Sulfuric acid causes severe burns. Use extreme care when handling.

Reagent③ : Dissolve DNPH in 3 ml of 44% sulfuric acid

※ This kit is sufficient for 100 assay points (50 samples, duplicate assay points).

【 PRINCIPLE OF THE ASSAY】



AsA in a given sample is converted to DHAsA by oxidizing agent. DHAsA is then derivatized with DNPH. Total vitamin C concentration (AsA + DHAsA) can be determined by the specific UV absorption of the DNPH derivative.

【 MATERIALS NOT SUPPLIED 】

- Micropipets
- Thermostat bath (37℃)
- Microplate reader (530 nm) or spectrophotometer※
  - ※ 100 µl cuvette (for spectrophotometer)
  - ※ Metaphosphoric acid (WAKO 135-12755 or Sigma M6288)
  - ※ Sulfuric acid (SA) (Sigma 339741)

【 SAMPLE PREPARATION 】

All operation should be performed on ice.

*Metaphosphoric acid solution necessary for sample preparation is not included in this kit.

「Tissue」

① Homogenize one volume of fresh tissue 13 volumes of 5.4% metaphosphoric acid. (Final: 5%)
② Centrifuge at 10,000 x G for 15 minutes at 4℃
③ Remove clear supernatant carefully from pellet. Store supernatant at -20℃ until ready for use.

「Plasma」

① Add equal amount of 10% metaphosphoric acid to plasma (Final: 5%), and stir this solution.
② Centrifuge at 10,000 g for 15 minutes at 4℃
③ Remove clear supernatant carefully from pellet. Store supernatant at -20℃ until ready for use.

【 ASSAY PROTOCOL 】

	Sample ・ Control (S) (C)	Standard (Std)
	Sample 200 µl	Standard 200 µl
Reagent①	12 µl	12 µl
Incubate 3-5 minutes at room temperature		
Reagent②※1	70 µl	70 µl

Total volume is 282 µl. Dispense into tubes as follows.

Solution A / Solution B

	Sample (S)	Control (C)	Standard (Std)
	Solution A 80 µl	Solution A 80 µl	Solution B 80 µl
Reagent③	20 µl	----	20 µl
Incubate 3 hours at 37℃※2			
85% SA※3	100 µl	100 µl	100 µl
Reagent③	----	20 µl	----
Incubate 30 minutes at room temperature			

Dispense 100 µl into each well of a 96 well plate.
Read absorbance at 530 nm on microplate reader.

Or, read samples with spectrophotometer (530 nm) and 100 µl cuvette.

Real absorbance is calculated as the absorbance value of the Sample minus the absorbance value of control (C). Correction of Standards (Std) is not necessary.

※１ Reagent ② will contain insoluble material. Just before use, shake reagent② well. Use as suspended solution. Insoluble material will not affect assay results. Note: Addition of Reagent ① to sample will cause purple color. Following addition of Reagent ② incubate until purple color fades.
※２ Red insoluble material will settle.
※３ Resuspend insoluble material by shaking.

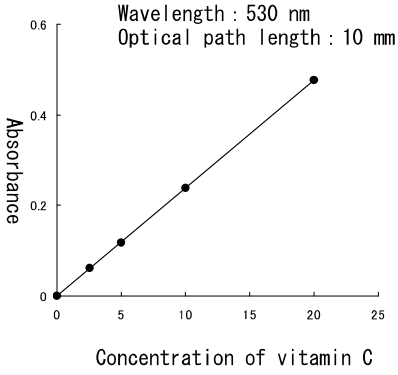
【 STANDARD CURVE PROTOCOL 】
Dilute vitamin C standard stock solution in reagent④ conforming to this protocol. Use this diluted solution as standard (std) conforming to assay protocol.

No.	Vitamin C standard stock solution	Reagent④	Concentration
1	----	400 μl	0
2	5 μl	395 μl	2.5 μg/ml
3	10 μl	390 μl	5 μg/ml
4	20 μl	380 μl	10 μg/ml
5	40 μl	360 μl	20 μg/ml

A typical standard curve following this protocol is shown below.

Standard curve

BECKMAN DU^{R}530 Life Science UV/Vis Spectrometer
Wavelength : 530 nm
Optical path length : 10 mm



【 OPERATION PRECAUTION 】
Hemolysis causes assay errors.
Anticoagulants such as the EDTA do not affect measurements.

【 STORAGE 】
Storage: store Frozen
Expiration date: 2 years after production

【 REFERENCES 】
Daniel W.B., Gladys E, James E.M. : Clinica Chimica Acta, 44, 47-52 (1973)

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