Vitamin C
HPLC Assay

Catalog Number: VIC31-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019Fax: 617-419-1110
www.EagleBio.com

1. Intended purpose

The Eagle Biosciences Vitamin C HPLC Assay kit is intended for the quantitative determination of vitamin C in Li-heparinate plasma and serum. The Vitamin C HPLC Assay kit is for research use only and not to be used in diagnostic procedures.

2. Introduction

The disease, which is called "scurvy", was known already 1550. The symptoms were known also by Greeks and Romans. In the 15th to 17th century more sailors died of scurvy than of any other circumstances. The food provided on board contained nearly no vitamin C containing goods. In the 16th century the importance of vitamin C in healing scurvy was discovered.

Vitamin C is a strong reducing agent. The oxidation of vitamin C leads to dehydroascorbic acid via a radicalic intermediate, in vivo. The three forms constitute a reversible redox-system. The most important function of ascorbic acid is the catalization in the biosynthesis of collagen. It is important for the de novo synthesis of bone, cartilage and teeth and wound healing. Vitamin C is needed for the synthesis of noradrenalin. Vitamin C plays an important role as antioxidant. It protects other molecules and cell structures from free radicals and reactive oxygen species. Ascorbic acid promotes the resorption of iron in the intestine and reduces the production of nitrosamines which might be carcinogenic.

The Eagle Biosciences Vitamin C HPLC Assay kit makes it possible to determine the vitamin in an easy, fast and precise method. The Vitamin C HPLC Assay kit contains all reagents ready to use for preparation and separation of the samples with exception of the column (IC2900rp) and the controls (IC2900ko). Both can be supplied by Eagle Biosciences. Beside the complete test kit it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of the Vitamin C HPLC Assay kit are strictly intended for research use only and are not to be used for diagnostic procedures.
- Test kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange Vitamin C HPLC Assay kit components from different lots.
- Calibrator and controls contain human blood. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- The precipitating reagent contains acid and has to be handled carefully. It is corrosive and

causes burns. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.

- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

Article no.	Component	Designation	Amount
IC2900lm	ELU	Mobile phase	1000 ml
IC2900ka	CAL	Calibrator, (lyoph. 0.25 ml)	8 vial
IC2900fr	PREC	Precipitation reagent	1 vial
IC2900rl	RECON	Reconstitution solution	25 ml

5. Additional special equipment

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with UV-detector
- HPLC column Vitamin C (IC2900rp)
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in **0.25 ml** reconstitution solution (RECON). The reconstituted calibrator is not stable and cannot be reused. The concentration of vitamin C might have minor changes from lot to lot.
- Reconstitute the precipitation reagent (PREC) in 25 ml reconstitution solution (RECON) in a ultrasonic bath (10 min.). The precipitation reagent is then stable for 3 month at 2-8 °C.
- All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

7. Specimen

- Venous fasting blood can be used in this test system. We recommend using Lithium-heparinate plasma because vitamin C is stabilized. Commercial available sample tubes (e.g. Sarstedt S-Monovette LH) could be used. The best stability is achieved when a 7.5 ml tube is filled with 2 ml of blood.
- Vitamin C is highly sensitive against oxidation; therefore samples should be stabilized immediately after arrival in the laboratory. For stabilization, add the precipitation reagent as indicated under "8. Sample Preparation".
- Plasma or serum, containing the precipitating reagent is stable for 24 h at 2-8°C. After centrifugation the supernatant is stable for at least 1 month at -20°C.

8. Procedure

Principle of the method

For the determination of vitamin C a precipitation step, which removes high molecular substances is performed first. After centrifugation the supernatant is injected into the HPLC system. The isocratic separation via HPLC at 30°C uses a "reversed phase" column. One run lasts 12 minutes. The chromatograms are recorded by a UV-detector. The quantification is performed by the delivered plasma calibrator; the concentration is calculated via integration of the peak areas, resp. peak heights.

Sample preparation

1. Pipette into 1.5 ml reaction tubes:

   200 µl sample, CAL or CTRL

   +

   200 µl PREC

2. Vortex briefly. Leave the tubes for **10 minutes at 2-8°C** and centrifuge at 10.000g for 10 minutes.

3. Inject **20 µl** of the supernatant into the HPLC-system

4. The supernatant is stable in the dark for at least 24 hours at 2-8°C.

Chromatographic conditions

Column material:	Bischoff Prontosil AQ, 5 µm
Column dimension:	125 mm x 4 mm
Flow rate:	0.75 ml/min
UV-detection:	254 nm
Injection volume:	20 µl
Running time:	12 min
Temperature:	30 °C

We recommend using a guard-column to enlarge lifetime of the HPLC column.

Treatment of the HPLC column

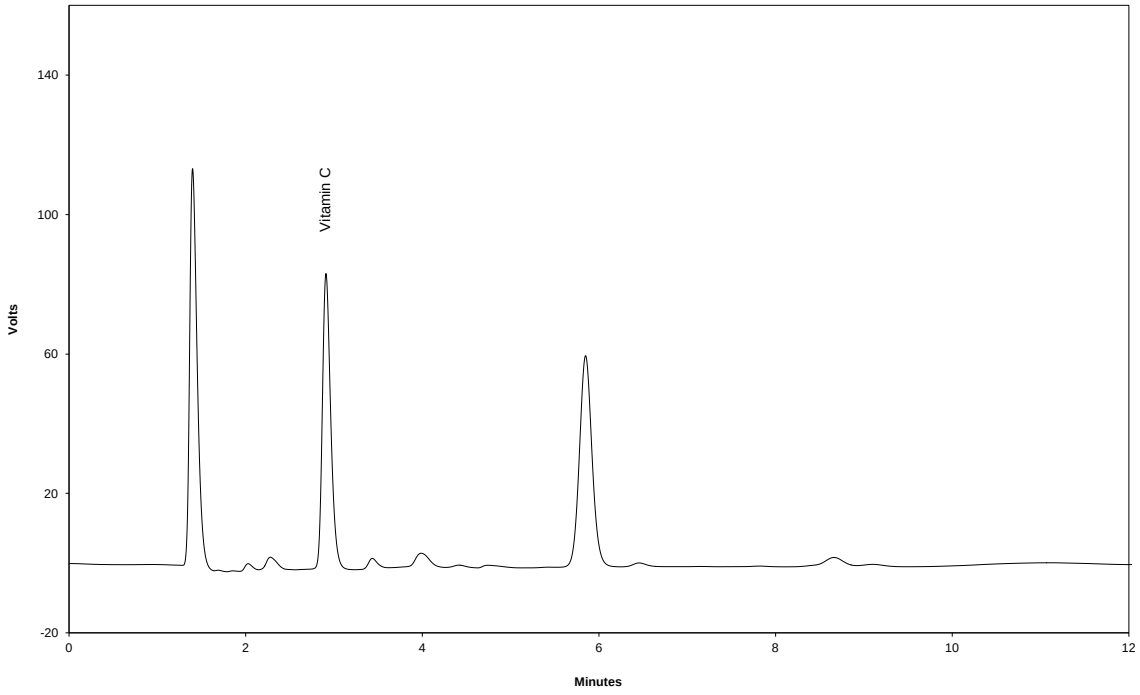
After the analysis the column should be flushed with 15 ml deionized water (1 ml/min) and stored in 50% methanol in deionized water (v/v) (approx. 15 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with ca. 30 ml mobile phase (ELU).

9. Calculation of analytical results

Calculation

$$\text{Conc. sample} = \frac{\text{peak area patient} * \text{conc. calibrator}}{\text{peak area calibrator}}$$

Typical chromatogram



10. Internal quality control

Reference intervals

4 – 21 mg/l

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Precision and reproducibility

Intra-Assay CV:	1.4 % (6.5 mg/l)	[n = 6]
	1.3 % (18.1 mg/l)	[n = 6]
Inter-Assay CV:	1.5 % (6.5 mg/l)	[n = 6]
	2.3 % (17.6 mg/l)	[n = 6]

Linearity

up to 250 mg/l

Detection limit

0.15 mg/l

Recovery

100.4 %

12. Limitations of the method

EDTA-blood should not be used in the Vitamin C HPLC Assay kit.

13. Disposal

The mobile phase (ELU) and the precipitation solution (PREC) could be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution. (**Important:** Reaction will produce heat, be careful). Please refer to the appropriate national guidelines.

14. Troubleshooting

Problem	**Possible reason**	**Solution**
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp
No peaks	Injector is congested	Check Injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the

		system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp

Continue baseline is drifting	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flowcell is dirty	Clean flow cell

15. Literature references

- Hultqvist M. et al. (1997). Plasma concentrations of vitamin C, vitamin E and/or malondialdehyde as markers of oxygen free radical production during hemodialysis. Clin Nephrol 47; 37-46.
- Falch J.A. (1998). Low levels of serum ascorbic acid in elderly patients with hip fracture. Scand J Clin Lab Invest 58; 225-228.
- Ballmer et al. (1994). Depletion of plasma vitamin C but not of vitamin E in response to cardiac operations. J Thorac Cardiovasc Surg 108; 311-320.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences at info@eaglebio.com or at 866-411-8023.