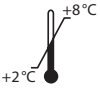
Manual

Vitamin C HPLC Kit

For the determination of vitamin C in Li-heparine plasma

Valid from 2015-12-30

REF **KCR2900**



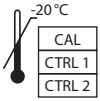


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Table of Contents

1. INTENDED USE 2

2. SUMMARY AND EXPLANATION OF THE TEST 2

3. PRINCIPLE OF THE TEST 2

4. MATERIAL SUPPLIED 3

5. MATERIAL REQUIRED BUT NOT SUPPLIED 3

6. STORAGE AND PREPARATION OF REAGENTS 4

7. PRECAUTIONS 4

8. SPECIMEN COLLECTION AND PREPARATION 4

9. ASSAY PROCEDURE 5

Procedural notes 5
Test procedure 5
Chromatographic conditions 6

10. TREATMENT OF THE COLUMN 6

11. RESULTS 6

Calculation 6
Typical chromatogram 7

12. LIMITATIONS 7

13. QUALITY CONTROL 7

Reference range 7
Controls 7

14. PERFORMANCE CHARACTERISTICS 8

Precision and reproducibility 8

15. DISPOSAL 8

16. TROUBLESHOOTING 8

17. REFERENCES 10

18. GENERAL NOTES ON THE TEST AND TEST PROCEDURE 10

1. INTENDED USE

This HPLC application is intended for the quantitative determination of vitamin C in plasma. For research use only. Not for use in diagnostic procedures.

2. SUMMARY AND EXPLANATION OF THE TEST

In the 15th to 17th century, more sailors died of scorbut than of any other disease. The food provided on board contained nearly no vitamin C. In the 16th century, the importance of vitamin C supplied by citrus fruits in healing scorbut was discovered.

Ascorbic acid (vitamin C) is a strong reducing substance. The oxidation of vitamin C leads over a radical intermediate to dehydroascorbic acid *in vivo*. The three forms mentioned constitute a reversible redox-system.

Ascorbic acid plays an important role in hydroxylation reactions, i. e. in the synthesis of collagen. So it is rather important for the *de novo* synthesis of bone, cartilage and tooth, and for the healing of wounds. Vitamin C is needed for the production of noradrenalin. Another important role of vitamin C is its antioxidant capability, e. g. protection of other substances from oxidative damage. Ascorbic acid promotes the resorption of iron in the intestine. In addition, it reduces the production of nitrosamines which might cause cancer.

3. PRINCIPLE OF THE TEST

The first step in the vitamin C determination is precipitation of the higher molecular components. After their removal by centrifugation, the supernatant is injected into the HPLC system.

The vitamin C analysis via HPLC follows an isocratic method at 30 °C using a reversed phase column. One run lasts 12 minutes. The chromatograms are recorded by an UV detector. The quantification is performed with the delivered calibrator. The concentration is calculated via integration of the peak areas/heights by the external standard method.

Summary

This HPLC application allows the quantitation of vitamin C in an easy, fast, and precise way. The kit contains all reagents necessary for sample preparation and separation in ready-to-use form except the column.

As for many other parameters, the advantage of HPLC analytics is the simultaneous handling of many analytes in a single test. The HPLC complete system enables even laboratories without experience in high performance liquid chromatography to use this technique for clinical chemical routines quickly and precisely. Mostly, a one-

point calibration is sufficient for calibrating the test system – unlike immunoassays with up to 6 calibrators per test. It is possible to automate the sample application and calculation of the results so that even higher number of samples can be handled nearly without control. With short test series, the one-point calibration is much more economic than 6-point calibration for immunoassays.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KCR2900	MOPHA	Mobile phase	1000 ml
KCR2900	CAL	Calibrator (250 µl lyoph.; see label for concentration)	8 vials
KCR2900	PREC	Precipitation reagent (lyoph.)	1 vial
KCR2900	RECSOL	Reconstitution solution	2 x 15 ml
KCR2900	CTRL1 CTRL2	Control 1 and 2 (250 µl lyoph.; see specification data sheet for concentration)	2 x 3 vials

For reorders of single components, use the catalogue number followed by the label as product number.

The HPLC column (KCR2900RP) as well as individual components can be ordered separately from Immundiagnostik. Please ask for the price list of the individual components.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- 1.5 ml reaction tubes (e. g. Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with UV detector
- Reversed phase C_{18} column
- Vortex

* Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6. STORAGE AND PREPARATION OF REAGENTS

- **The lyophilised calibrator** (**CAL**) is stable at **-20 °C** until the expiry date stated on the label. Before use, the CAL has to be reconstituted with **250 µl ultra pure water**. The concentration of vitamin C slightly changes from lot to lot, the exact concentration is stated on the label. **Calibrator** (reconstituted CAL) **is not stable and cannot be stored.**
- **The lyophilised controls 1 and 2** (**CTRL 1 and CTRL 2**) are stable at **-20 °C** until the expiry date stated on the label. Before use, they have to be reconstituted with each **250 µl ultra pure water**. The concentration of vitamin C slightly changes from lot to lot, the exact concentration is stated on the specification data sheet. **Controls** (reconstituted CTRL 1 and 2) **are not stable and cannot be stored.**
- **The lyophilised precipitation reagent** (**PREC**) is stable at **2–8 °C** until the expiry date stated on the label. Before use, the PREC has to be reconstituted with **25 ml reconstitution solution** (RECSOL) for ~10 min. **Precipitation reagent** (reconstituted PREC) is stable at **2–8 °C** for **3 months.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

7. PRECAUTIONS

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The precipitation reagent (PREC) contains acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
- Reagents should not be used beyond the expiration date stated on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

Lithium-heparine plasma is suitable for this test system. Commercially available sample tubes (e. g. Sarstedt S-Monovette LH) should be used. A sample treated in this way is stable for 24 hours at 4 °C.

Vitamin C is highly sensitive against oxidation; therefore, samples should be stabilized immediately after arrival in the laboratory. For stabilisation, the precipitation reagent must be added and centrifuged (see assay procedure). Afterwards, the supernatant is stable for at least 8 weeks at -20 °C.

After thawing the samples, the analysis should be done as soon as possible.

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- The assay should always be performed according the enclosed manual.

Test procedure

Pipet each **200 µl sample**, **calibrator or control 1 or 2** into an 1.5 ml reaction tube.
Add each **200 µl precipitation reagent** and mix well.
Incubate for **10 min** at **2–8 °C**.
Centrifuge for **10 min** at **10 000 *g*** and take the supernatant. The supernatant is stable for at least 24 hours at room temperature if kept in the dark.
Inject **20 µl** supernatant into the HPLC system.

Chromatographic conditions

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

It is recommended to use a guard column to extend column life.

10. TREATMENT OF THE COLUMN

After analysis, the column should be flushed with 30 ml ultra pure water (1 ml/min) and stored in 50% methanol in water (~30 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with ~ 30 ml mobile phase (MOPHA).

11. RESULTS

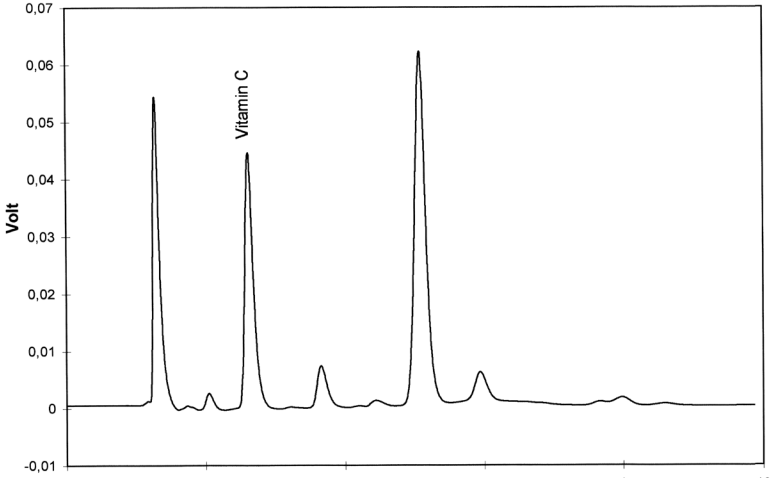
Calculation

$$\text{Sample concentration (nmol/l)} = \frac{\text{Peak height sample} \times \text{calibrator concentration*}}{\text{Peak height calibrator}}$$

* see label

Tip: Alternatively, the peak area instead of the peak height can be used for quantification.

Typical chromatogram



12. LIMITATIONS

EDTA-blood is not suitable for this test system and should not be used.

13. QUALITY CONTROL

Reference range

We recommend each laboratory to establish its own reference range.

Controls

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

14. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intraassay CV

5.6 % (4.4 mg/l) [n=6]
4.1 % (18.8 mg/l) [n=6]

Interassay CV

8.8 % (4.4 mg/l) [n=8]
5.9 % (18.6 mg/l) [n=8]

15. DISPOSAL

The mobile phase (MOPHA) and precipitation reagent (PREC) can be neutralized to neutral pH with NaOH and disposed as a salt solution.

Important: Reaction will produce heat, be careful!

Please refer to the appropriate national guidelines.

16. TROUBLESHOOTING

Problem	Possible cause	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

Problem	Possible cause	Solution
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
	Auto sampler 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
	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 flow cell is dirty	Clean flow cell

17. REFERENCES

1. 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.
2. Falch J.A. (1998). Low levels of serum ascorbic acid in elderly patients with hip fracture. *Scand J Clin Lab Invest* **58**; 225-228.
3. 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.

18. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the kit package are for research use only.
- Reagents should not be used beyond the expiration date stated on kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

Used symbols:


Temperature limitation

Catalogue Number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet