Manual

IDK® Vitamin C

For colorimetric determination of vitamin C in Li-heparine plasma, serum and urine

Valid from 2019-05-23

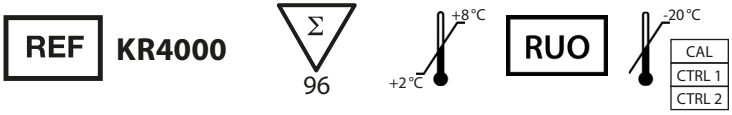

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Table of Contents

1. INTENDED USE

This colorimetric microtiter plate assay is suitable for the determination of vitamin C (ascorbic acid) in Li-heparine plasma, serum and urine.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Vitamin C (ascorbic acid), being a part of the antioxidative defense system, is found in both the cytosol and extracellular spaces. Depending on the concentration and the availability of transitional metals, it has antioxidative as well as prooxidative features. The antioxidative effect dominates, especially in extracellular space. Since it acts through formation of semi-dehydro-ascorbate and dehydro-ascorbate respectively, as an electron donor transferring hydrogen to acceptor substances by reversibility, ascorbic acid has strong reducing effects.

Vitamin C contributes to the antioxidative defense system in two different ways: it reacts with reactive oxygen species, especially peroxide radicals, and regenerates a-tocopherol (vitamin E). Vitamin C also has a pro-oxidative effect in combination with transition metals. It catalyses the reduction of Fe^{3+} to Fe^{2+}. The created bivalent iron ions react faster with H_2O_2. Therefore, the formation of OH• radicals is supported through the Haber-Weiss-Reaction.

Due to the very small concentration of free transition metals in biological tissues, the antioxidative features are predominant.

3. PRINCIPLE OF THE TEST

In serum and plasma vitamin C is found as ascorbic acid as well as its oxidized form, dehydro-ascorbate. Both forms are biologically active. In our vitamin C assay, an oxidation is induced prior to analysis so that both forms are measured. A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from the standard. The concentration of the sample is determined using the value obtained from calibrator and the blank value.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR4000	PREC	Precipitation reagent	20 ml
KR4000	SOL A	Reagent solution A	7 ml

KR4000	SOL B	Reagent solution B	1 ml
KR4000	SOL C	Reagent solution C	1 ml
KR4000	STOP	Sulfuric acid	20 ml
KR4000	CAL	Calibrator, lyophilised (see QC data sheet for concentrations)	4 x 250 µl
KR4000 KR4000	CTRL1 CTRL2	Control 1 and 2, lyophilised (see specification for ranges)	each 4 x 250 µl
KR4000	PLATE	Microtiter plate (MTP)	1 piece
KR4000	FOL	Microtiter plate coverfoil	2

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

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Precision pipettors and disposable tips to deliver 20–200 µl and 100–1000 µl
- Multi-channel dispenser or repeating dispenser
- 1.5 ml reaction tubes (e. g. Eppendorf)
- 15 ml tubes (e.g. Falcon)
- Horizontal microtiter plate shaker
- Centrifuge
- Vortex mixer
- Incubator for 37 °C
- Microtiter plate reader at 490–520 nm
- A suitable place mat when working with solution A, because solution A contains dye which cannot be cleaned off plastic surfaces

* Immundiagnostik AG recommends the use of ultrapure 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. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- The **lyophilised calibrator** (CAL) is stable at -**20 °C** until the expiry date stated on the label. The CAL must be reconstituted with **250 µl of ultrapure water**.

Allow the vial content to dissolve for 10 minutes at room temperature, and mix thoroughly by gentle inversion to insure complete reconstitution. **Calibrator** (reconstituted CAL) **is not stable and cannot be stored.**

- The **lyophilised controls** (CTRL) are stable at -**20 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification. Controls** (reconstituted CTRL) **are not stable and cannot be stored.**
- 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. SAMPLE PREPARATION AND STORAGE

Urine samples

Urine samples must first be **diluted 1:4** (e.g. 250 µl urine + 750 µl ultrapure water). This dilution factor must be taken into consideration when calculating the concentration.

Take 200 µl of the diluted sample and use it for the sample preparation explained below.

Serum and plasma samples

Serum and plasma samples are not to be diluted and used directly for the sample preparation explained below.

Storage

Attention: Samples should be kept in a cool and dark place. Samples can then be measured withing 24 hours after blood withdrawal. Samples are not stable at room temperature.

8. ASSAY PROCEDURE

Water is used as **blank** (zero standard).

We recommend to carry out the tests in duplicate.

Sample preparation

1.	Pipet 200 µl prepared sample, **calibrator** (reconstituted CAL), blank and **control 1** and **control 2** (reconstituted CTRL1 and CTRL2) into 1.5 ml reaction tubes and add 200 µl **precipitation agent** (PREC).
2.	Mix well.

3.	Centrifuge at 10 000 *g*, 30 minutes.

Test procedure

1.	The **working solution** must be **prepared directly before the test**: mix 10 volumes of **reagent solution A** (SOL A) with each 1 volume of **reagent solution B** and **reagent solution C** (SOL B and C); example for a whole plate: 6 ml SOL A plus each 600 µl SOL B and SOL C. **Please note**: **reagent solution A** contains dye which cannot be cleaned off plastic surfaces. It is therefore recommended to use a suitable place mat when working with reagent solution A.
2.	Add **2 x 100 µl** of the **supernatants** of **calibrator** (CAL), **blank**, **control 1** and **control 2** (CTRL1 and CTRL2) or **samples** into the **microtiter plate** (PLATE) wells in duplicates.
3.	Add **50 µl** of the freshly prepared **working solution** in the wells.
4.	Cover the **microtiter plater** (PLATE) with foil and incubate **for 3 h at 37 °C.**
5.	Add **150 µl** of **sulfuric acid** (STOP) in the wells.
6.	Shake **microtiter plate** (PLATE) on a horizontal shaker at **room temperature** (15 - 30 °C) **for 20 min** (without any foil cover). An orange precipitate can be formed. The precipitate can be dissolved by repeatedly (2-3 times) drawing up the solution with the pipette.
7.	Determine the absorption at 492 nm or 520 nm.

9. RESULTS

Linear regression is used to calculate the results. The blank must be specified with the value zero.

Please note: for the analysis of **urine samples** the used dilution factor has to be taken into consideration.

Please refer to the calibrator specification for the concentration of the calibrator (CAL).

Please refer to the control specification for the concentrations of controls (CTRL).

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

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.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=12

The repeatability was assessed according to CLSI guideline EP5-A2 with a Li-heparine plasma sample under constant parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [mg/l]	CV [%]
1	54.7	6.5

Reproducibility (Inter-Assay); n=42

The reproducibility was assessed according to CLSI guideline EP5-A2 with 2 Li-heparine plasma samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [mg/l]	CV [%]
1	27.1	7.1
2	40.3	7.9

12. PRECAUTIONS

- All reagents in the kit package are for research use only.

- 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.
- Sulfuric acid (STOP) is composed of sulfuric acid, which is a strong acid. It 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.
- Precipitating reagent (PREC) contains acid and 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 breath vapour and avoid inhalation.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- 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.

15. REFERENCES

1. Böhn U et al. (2003) Rationelle Diagnostik in der Orthomolekularen Medizin. Hippokrates Verlag, Stuttgart
2. Esteve MJ, Farre R, Frigola A, Garcia-Cantabella JM (1997) Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. J Chromatogr B Biomed Sci Apll. 24;688(2):345-9.
3. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry, 5th ed. Saunders: Philadephia, 2011

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