

Revised 3 Aug. 2011 rm (Vers. 2.1)

*This kit is intended for Research Use Only.**Not for use in diagnostic procedures.**Please use only the valid version of the package insert provided with the kit.***INTENDED USE**

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

**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 directly from the linear standard curve.

**MATERIAL SUPPLIED**

	Kit Components	Quantity
PREC	Precipitation reagent	15 ml
SOL A	Reagent solution A	7 ml
SOL B	Reagent solution B	1 ml
SOL C	Reagent solution C	1 ml
STOP	Sulfuric acid	20 ml
STD	4 Standards (lyophilized)	each 4 x 400 µl
CTRL1 and CTRL2	Control 1 and 2 (lyophilized)	each 4 x 400 µl
PLATE	Microtiter plate (MTP)	12 x 8 wells
FOL	Microtiter plate cover foil	2

**MATERIAL REQUIRED BUT NOT SUPPLIED**

- Bidistilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 20 - 200 µl and 100 - 1000 µl
- A multi-channel dispenser or repeating dispenser

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- 1.5 ml Eppendorf cups
- 15 ml Tubes (e.g. Falcon)
- Horizontal microtiter plate shaker
- Centrifuge suitable for 15 ml tubes at 10 000 x g
- Vortex-Mixer Incubator for 37 °C
- Microtiter plate reader at 490 - 520 nm (reference wave length 610 - 630 nm)
- A suitable place mat when working with solution A, because solution A contains dye which might be difficult to clean off plastic surfaces

#### PREPARATION AND STORAGE OF REAGENTS

All reagents are stable at 2-8 °C up to the expiry date stated on of the label.

**STD** (standards) and **CTRL** (controls) must be reconstituted with 400 µl bidist. water.

Allow the vial content to dissolve for 10 minutes. Reconstituted standards and controls are not stable.

**Please note:** Samples should be kept cool and light-protected. Samples can be measured within 24 hours after blood withdrawal.

#### 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.
- Stop Solution 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 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.
- Reagents should not be used beyond the expiration date shown on the kit label.

#### SAMPLE AND REAGENT PREPARATION

- Pipet 200 µl fresh collected Li-heparine-plasma sample, serum or STD (standards) and CTRL (controls) in Eppendorf cups, respectively, and add 200 µl PREC (precipitation reagent)
- Urine samples must be diluted 1:4 before analysis (e. g. 250 µl urine + 750 µl aqua dist.). The dilution factor must be considered when calculating the concentration.
- Vortex well
- Centrifuge at 10000 x g for 30 min

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**Preparation of the working solution:**

To run a complete microtiter plate:

Add 600 µl of each, Solution B and C (reagent solution B and C) to 6 ml of **Solution A** (reagent solution A).

To run assay more than once, prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

**Please note:**

**Solution A** contains dye which might be difficult to clean off plastic surfaces. It is therefore recommended to use a suitable place mat when working with **Solution A**.

**ASSAY PROCEDURE**

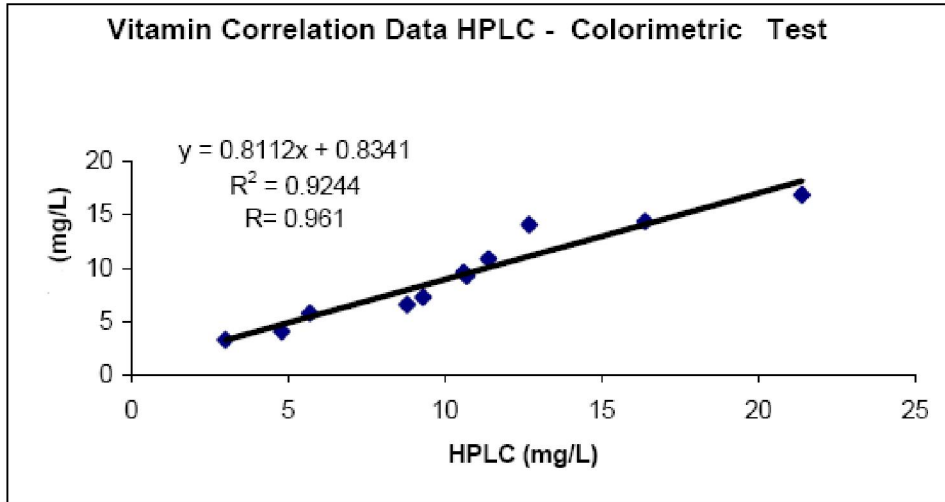
1. Add **2 x 100 µl** of the **supernatants** of **STD, CTRL** or **samples** into the microtiter plate wells in duplicates
2. Add **50 µl** of the freshly prepared **working solution** in the wells
3. Cover the microtiter plate with foil and incubate **for 3 h at 37 °C**
4. Add **150 µl** of **STOP (Stop Solution)** in the wells
5. Shake microtiter plate on a horizontal shaker at **room temperature 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.
6. Determine the absorption at 492 nm or 520 nm against 620 nm as a reference

**RESULTS**

A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from standard. The concentration of samples is determined directly from the linear standard curve.

It is recommended to use a point-to-point-calculation.

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**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 test package are for research use only.
- Do not use the reagents after the date of expiry stated on the label.
- Do not interchange different lot numbers of any kit component within the same as-say.
- 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. DRG can therefore not be held responsible for any damage resulting from wrong use.

**REFERENCES / LITERATURE**

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 Appl. 24;688(2):345-9.
3. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 4<sup>th</sup> ed. Saunders: Philadelphia, 2006:1107.

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