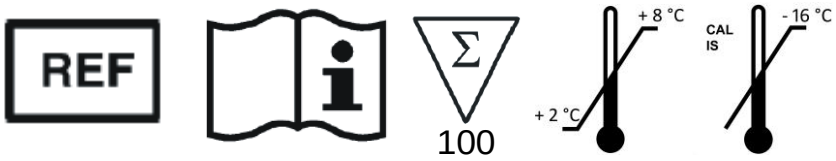
Manual

Ubiquinone

HPLC

For the determination of Ubiquinone (Coenzyme Q_{10}) in plasma, serum and EDTA-blood

Valid from 03.12.2019



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1. Intended purpose

The *ImmuChrom* Assay is intended for the quantitative determination of ubiquinone (coenzyme Q10) in plasma, serum and EDTA-blood. For research use by trained personnel in laboratories only.

2. Introduction

Ubiquinone is a coenzyme of every cell in the body. It was first isolated in the 50th from Prof. Green from Wisconsin. For his investigations concerning the function Prof. Mitchel received 1978 the Nobel price.

Ubiquinone is built up by a chinonic ring and an isoprenic sidechain. In humans ubiquinone can be synthesized and taken up by nutrition.

Ubiquinone has two different pysiological functions.

1.) Component of the energy metabolism

During the reduction of oxygen in the oxydative phosphorylation electrons are tranferred from NADPH to oxygen via 6 different redox systems. Ubiquinone is the less abundant redox system in the membrane of the mitochondria. Because of the low amount it is the speed controlling redox-system in the energy metabolism. Normally the amount of ubiquinone is sufficient, but with growing age and exposure to sunlight it is reduced to 50 %.

2.) Radical scavanger

Ubiquinone has a high amount of carbon doublebonds and therefore a higher potential of reduction than vitamin C or vitamin E. Thereby it is the first line of defense against free radicals. Even when other antioxidants are inert coenzyme Q10 reacts fast and sensitive against free radicals. Therefore ubiquinone is an optimal stabilizer of the ion channels of the membranes.

Applications:

- Determination of ubiquinone status
- Cardiovascular disease
- Carcinogenesis
- Aging
- Burnout syndrome

The ImmuChrom HPLC-application for ubiquinone (coenzyme Q_{10}) makes it possible to determine it in an easy, fast and precise method. The kits includes all reagents ready to use for preparation and separation of the samples with exception of the column (IC1700rp) and the controls (IC1700ko). Both can be supplied by ImmuChrom GmbH.

Beside the complete testkits it is possible to order all components separately. Please request our single component pricelist.

3. Warnings and precautions

All reagents of this kit are strictly intended for research use only.

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

Do not interchange kit components from different lots.

Testkit and column are concerted . Using alternative columns might cause in unsufficient separation, resulting in false high results. The given test characeristics might not be fulfilled.

Calibrator and controls contain human plasma. 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 plasma based reagents in this kit must be handled as though capable of transmitting infection.

The internal standard, extraction solution and mobile phase contain organic solvents and have to be handled carefully. Organic solvents are highly flammable and toxic if inhaled or contact the skin. They 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. Material delivered in the test package

Article no.	Component	Designation	Amount
IC1700lm	ELU	Mobile phase	1000 ml
IC1701ka	CAL	Calibrator, (lyoph. 500 µl)	5 vial
IC1701io	IS	Internal standard with oxidationreagent	25 ml
IC1701re	RECON	Reconstitution solution	5 ml
IC1701sb	STAB	Stabilisation solution	2,5 ml
IC1701fr	PREC	Precipitation solution	25 ml

5. Additional special equipment

- 1.5 ml reaction vials
- Centrifuge
- Various pipettes
- HPLC with UV-detector
- HPLC column Ubiquinone (IC1700rp)
- Vortex mixer

6. Reagent preparation

Reconstitute the **calibrator (CAL)** in **0.5 ml** reconstitution solution (RECON), divide the calibrator in several portions and store them at <-16 °C. The resuspended calibrator is stable for at least 14 days. Avoid repeated freeze-thaw circles. The concentration of ubiquinone might have minor changes from lot to lot.

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

7. Specimen

EDTA-plasma, EDTA blood and serum could be used.

The samples are stable in the dark at 20-25 °C for 48 hours, at 2-8°C for 1 week. For longer time samples should be stored at <-16 °C.

8. Procedure

Principal of the method

For the determination of ubiquinone (coenzyme Q_{10}) the sample is oxidized and the total ubiquinone is meassured. Coenzyme Q10 is extracted out of the sample. The internal standard reduces the variation in the sample treatment. After centrifugation the upper layer is injected into the HPLC system.

The isocratic separation via HPLC at 30°C using a „reversed phase“ column needs 15 minutes. The chromatograms are recorded by a UV-detector. The quantification is performed by the delivered calibrator and the concentration is calculated by the “internal standard method” via integration of the peak areas resp. peak heights.

Sample preparation

Pipette into 1.5 ml tubes:

250 µl PREC

+

200 µl sample, CAL or CTRL

+

25 µl STAB

Mix well on a vortex mixer (10 s).

Add **250 µl** IS OX

Mix well on a vortex mixer (10 s).

Leave it for **10 min** at **20-25 °C**. Mix for **2 min** on a vortex mixer (Extraction).

Leave it for **10 min** at **2-8 °C** and centrifuge for 5 min at 10000 g

Take **100 µl** of the upper layer and add **200 µl** ELU

Leave it for **10 min** at **2-8 °C** and centrifuge for 5 min at 10000 g

Inject **60 µl** of the upper phase into the HPLC-system
(The extracted supernatant is stable for 48 h at 2-8 °C.)

Chromatographic settings

Column material:	Ubiquinone column (IC1700rp)
Column dimension:	125 mm x 4 mm
Flow rate:	0.8-1.2 ml/min
UV-detection:	275 nm
Injection volume:	60 µl
Running time:	15 min
Temperature:	30 °C

Treatment of the HPLC-column

The column is left in mobile phase after analysis. Before use, equilibrate the system with approx. 20 ml mobile phase (ELU) (1 ml/min).

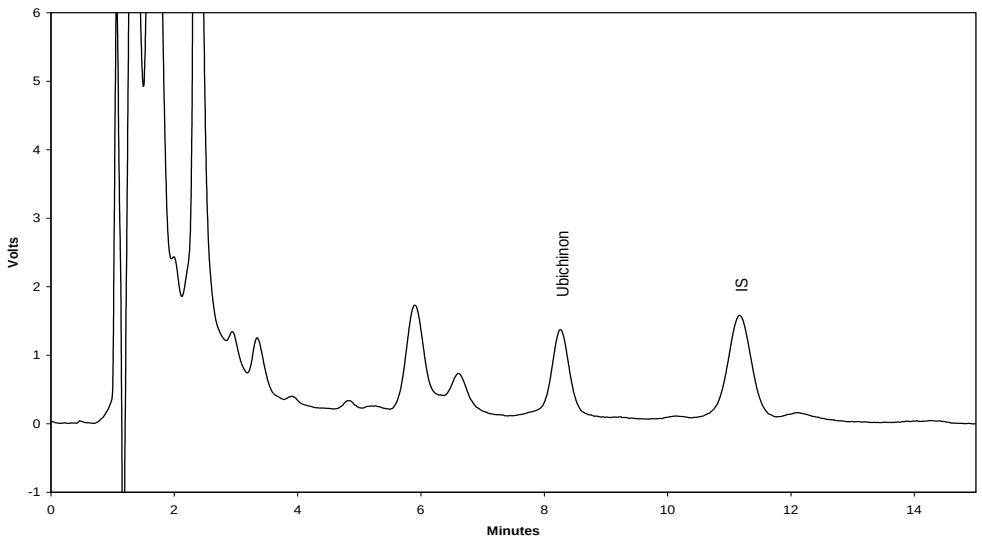
9. Calculation of analytical results

Calculation

$$\frac{\text{peak area patient} \cdot \text{concentration of the standard}}{\text{peak area IS patient}} * F = \text{concentration patient sample}$$

$$F = \frac{\text{Peak area IS of the calibrator}}{\text{Peak area analyte of the calibrator}}$$

Typical chromatogram



10. Internal quality control

Reference intervals

EDTA-plasma: **0.8 – 1.4 µg/ml**

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

Lit: Mortensen S. A. et al: (1997) Molec.Aspects Med. 18:137-144

11. Validation data

Precision and reproducibility

Intra-Assay CV:	< 5 %	[n = 6]
Inter-Assay CV:	< 7 %	[n = 6]

Linearity

up to 20 µg/ml

Detection limit

0.02 µg/ml

Recovery

98.4 %

12. Limitations of the method

In rare whole blood samples an overlapping of the internal standard peak could occur. The amount of extraction varies slightly from sample to sample. If the internal standard peak is 1.4 fold higher than in the calibrator sample we recommend to classify these sample as unplausible.

13. Disposal

The mobile phase (ELU), precipitation solution (PREC), internal standard (IS), and stabilization solution (STAB) must be disposed as non-halogenated solvent.

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
	Detectorlamp is altered	Change lamp
No peaks	Injector is congested	Check Injector
Doublepeaks	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

Mortensen S. A. et al: (1997) Molec.Aspects Med. 18:137-144