Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Manual

Coenzyme Q_{10} HPLC Kit

For the determination of coenzyme Q_{10} (ubiquinone) in EDTA-whole blood, serum and EDTA-plasma

Valid from 2022-09-05

REF **KC1700**

Σ 100

+2 °C … +8 °C

-20 °C

CAL
INT STD
CTRL 1
CTRL 2

IVD

CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This HPLC application is intended for the quantitative determination of coenzyme Q_{10} (ubiquinone) in EDTA-whole blood, serum and EDTA-plasma. For *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Ubiquinone was first isolated in the 50s from the group of Prof. Green (Wisconsin). The function was investigated by Prof. Mitchel, who received the Nobel price for his research on the oxydative phosphorylation pathway.

Ubiquinone is a coenzyme which is represented in every cell and the whole metabolism. It 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
2) Radical scavanger

During the reduction of oxygen in the oxydative phosphorylation 3 mol ATP are generated. In this reduction 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 who is controlling the 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 %.

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. Therefore ubiquinone is an optimal stabilizer of the ion channels of the membranes.

Indications

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

3. PRINCIPLE OF THE TEST

The first step in determining coenzyme Q_{10} includes sample preparation. Therefore the internal standard is added to the calibrator, controls and samples. Higher molecular substances are removed by precipitation and centrifugation.

The coenzyme Q_{10} in the supernatant is then extracted by an organic solvent, which is evaporated afterwards. The residue is resuspended in ethanol p.a. Afterwards, 100 µl of the resuspended solution are injected into the HPLC system.

The separation via HPLC follows an isocratic method at 30 °C using a reversed phase column. One run lasts 15 minutes. The chromatograms are recorded by a UV detector. The quantification is performed with the delivered calibrator; the concentration is calculated via integration of the peak areas/heights by the internal standard method.

Summary

The kit includes all reagents for preparation and separation of the samples, 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
K 0005.15	RECSOL	Reconstitution solution	15 ml
KC1700	MOPHA	Mobile phase (important: do not recirculate)	1 000 ml
	CAL	Calibrator; (lyophilised, see specification data sheet for concentration)	4 x
	INT STD	Internal Standard; ready-to-use	110 ml
	DIL	Dilution solution	85 ml
	EXTSOL	Extraction solution	220 ml
	ETHANOL	Ethanol p.A.	20 ml
	CTRL 1	Control 1; lyophilised	4 x
	CTRL 2	Control 2; lyophilised	4 x

The HPLC column (KC1700RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC1700VS) are highly recommended. These and also the pre-column holders (KC1700VH) can also be ordered from Immundiagnostik.

In addition to the complete kits, all components can also be ordered separately. Please ask for our single component price list.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- 10 ml screw capped glass vials (e. g. Pyrex)
- Centrifuge
- Vortex
- Various pipettes (100 µl, 1 000 µl)
- HPLC with UV detector
- Reversed phase C_{18} column
- Evaporation unit

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 **500 µl** reconstitution solution (**RECSOL**). The concentration of coenzyme Q_{10} slightly changes from lot to lot, the exact concentration is stated on the label. Aliquots of the **calibrator** (reconstituted CAL) can be stored at **-20 °C** for **14 days. Avoid repeated thawing and freezing.**
- 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** reconstitution solution (**RECSOL**). The concentration of coenzyme Q_{10} slightly changes from lot to lot, the exact concentration is stated on the specification data sheet.
- The internal standard (**INT STD**) is stable at **-20 °C** until the expiry date stated on the label.
- 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. SPECIMEN COLLECTION AND PREPARATION

EDTA-whole blood, serum or EDTA-plasma are suited for this test system.

The samples are stable for 1 day at 2–8 °C in the dark. For longer storage, samples should be frozen at -20 °C.

8. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- The assay should always be performed according the enclosed manual.
- Bring dilution solution (**DIL**) to room temperature before use to dissolve precipitations.

Test procedure

Pipet each **200 µl sample**, **calibrator or control 1 or 2** into a 10 ml screw capped glass reaction tube.
Add each **800 µl dilution solution** (DIL), **mix well** (vortex for 10 s).
Add each **1 ml internal standard** (INT STD), **mix well** (vortex for 10 s).
Add each **2 ml extraction solution** (EXTSOL).
Vortex for **2 min**.
Centrifuge for **10 min** at **3 000 *g***.
Take the **upper layer** and **evaporate** it. Resuspend the residue in **150 µl ethanol p. a.** (ETHANOL).
Inject **100 µl** into the HPLC system.

Chromatographic conditions

Column material:	Bischoff Prontosil AQ; 5 µm
Column dimension:	125 mm × 4 mm
Flow rate:	0.8–1.2 ml/min
	Please refer to the quality certificate of the column
UV detection::	275 nm
Temperature:	30 °C
Injection volume:	100 µl
Running time:	15 min

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

Use mobile phase (MOPHA) for autosampler and injection valve wash.

9. TREATMENT OF THE COLUMN

The column can be stored in the mobile phase (**MOPHA**) after the analysis.

Before use, the system should be equilibrated with 50 ml mobile phase (**MOPHA**): Run first 20 ml without column, and then the remaining 30 ml with integrated column.

10. RESULTS

Calculation

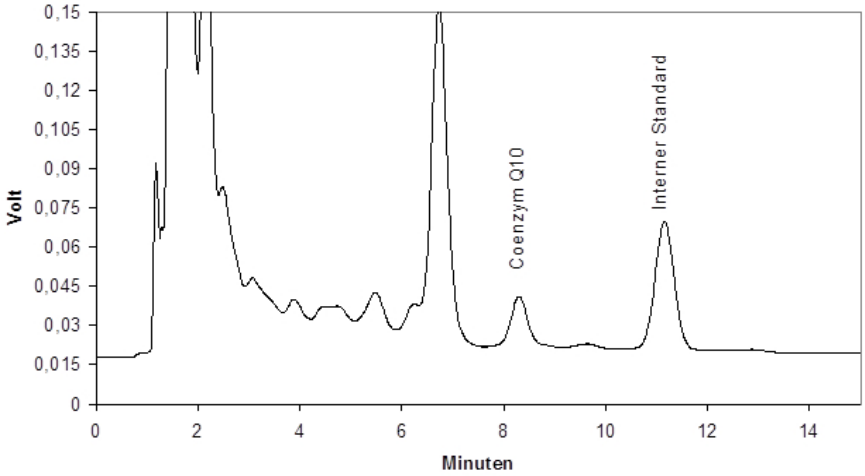
$$\frac{\text{Peak height sample} \times \text{concentration of the calibrator*}}{\text{Peak height internal standard of the sample}} \times F = \text{sample concentration}$$

$$F = \frac{\text{Peak height interal standard of the calibrator}}{\text{Peak height calibrator}}$$

* see label

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

Typical chromatogram



11. QUALITY CONTROL

Reference range

EDTA-whole blood: 0.67–0.99 µg/ml (mean ± 2 SD)

EDTA-plasma: 0.83–1.43 µg/ml (mean ± 2 SD)

We recommend each laboratory to establish its own reference range. The above mentioned values are only for orientation and may vary from other published data.

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 patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay CV

4.4 % (0.66 µg/ml) [n = 12]

Inter-Assay CV

6,6 % (0,31 µg/ml) [n = 10]

4,5 % (0,89 µg/ml) [n = 10]

Linearity

up to 10 µg/ml

Detection limit

0.02 µg/ml

13. DISPOSAL

The mobile phase (**MOPHA**), extraction solution (**EXTSOL**), internal standard (**INT STD**), and ethanol p. a. (**ETHANOL**) must be disposed as non-halogenated solvent. Please refer to the appropriate national guidelines.

14. 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

15. PRECAUTIONS

- For *in vitro* diagnostic 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.
- Reagents should not be used beyond the expiration date shown on kit label.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

16. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the kit package are for *in vitro* diagnostic 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 medical 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.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

Used symbols:


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivatives or human blood

Consult specification data sheet

Unique Device Identification


Medicinal substance

Catalogue number

To be used with

Contains sufficient for <n> tests


Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin