EAGLE BIOSCIENCES

Ubiquinone (Coenzyme Q_{10}) HPLC Assay

Catalog Number: Q1031-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019 Fax: 617-419-1110
www.EagleBio.com

1. Intended purpose

The Eagle Biosciences Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit is intended for the quantitative determination of ubiquinone (coenzyme Q10) in plasma, serum and EDTA-blood. The Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit is for research use only and should not be used for diagnostic procedures.

2. Introduction

Ubiquinone is a coenzyme of every cell in the body. Ubiquinone is made up by a chinonic ring and an isoprenic side chain. In humans ubiquinone can be synthesized and taken up by nutrition. Ubiquinone has two different physiological functions.

1.) Component of the energy metabolism

During the reduction of oxygen in the oxidative phosphorylation, electrons are transferred 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 scavenger

Ubiquinone has a high amount of carbon double bonds and therefore a higher potential of reduction than either 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.

The Eagle Biosciences Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit makes it possible to determine ubiquinone in an easy, fast and precise method. The Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit 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 Eagle Biosciences. Besides the complete test kit, it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of this Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit are strictly intended for research use only.
- Test kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange the Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit components from different lots.
- 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 serum 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 breathe 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. Materials Provided

Article no.	Component	Designation	Amount
IC1700lm	ELU	Mobile phase	1000 ml
IC1700ka	CAL	Calibrator, (lyoph. 500 µl)	5 vial
IC1700is	IS	Internal standard	110 ml
IC1700re	RECON	Reconstitution solution	5 ml
IC1700ex	EXT	Extraction solution	220 ml
IC1700vl	DIL	Dilution solution	85 ml
IC1700et	ETHA	Ethanol p.A.	20 ml

5. Additional Special Equipment

- 10 ml screw capped glass vials (Pyrex)
- Centrifuge
- Various pipettes
- HPLC with UV-detector
- HPLC column Ubiquinone (IC1700rp)
- Evaporation unit
- 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 -20 °C. The re-suspended 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.
- The dilution solution (DIL) might show crystallization during storage at 2-8°C. Before use re-dissolve the crystals at room temperature or at 37°C in a water bath.
- All other test reagents of the Ubiquinone (Coenzyme Q_{10}) HPLC Assay kit 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 2-8°C for 1 week. For longer time samples should be stored at -20 °C.

8. Procedure

Principal of the method

For the determination of ubiquinone (coenzyme Q_{10}) a precipitation step which removes high molecular substances is performed first. The internal standard reduces the variation in the sample treatment. After centrifugation the supernatant is mixed with an extraction solution and transferred to the organic phase. The organic solvent is evaporated and the sample is re-suspended in ethanol p.A. and 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

1. Pipette into 10 ml screw cap tubes:

   200 µl sample, CAL or CTRL

   +

   800 µl DIL

   (**Important:** During storage at 2-8°C crystallization might occur. Re-suspend the crystals at room temperature or at 37°C in a water bath before use)

2. Mix well on a vortex mixer (10 s).
3. Add **1 ml** IS
4. Mix well on a vortex mixer (10 s).
5. Add **2 ml** EXTR
6. Mix for **2 min** on a vortex mixer and centrifuge for 10 min at 3000 g
7. Remove 1.5 ml from the upper layer and evaporate to dryness.
8. Resuspend the residue in 150 µl ETHA
9. Inject **50 µl** of the re-suspended sample into the HPLC-system

Chromatographic settings

Column material:	Bischoff Prontosil AQ, 5 µm
Column dimension:	125 mm x 4 mm
Flow rate:	0.8-1.2 ml/min
UV-detection:	275 nm
Injection volume:	100 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).

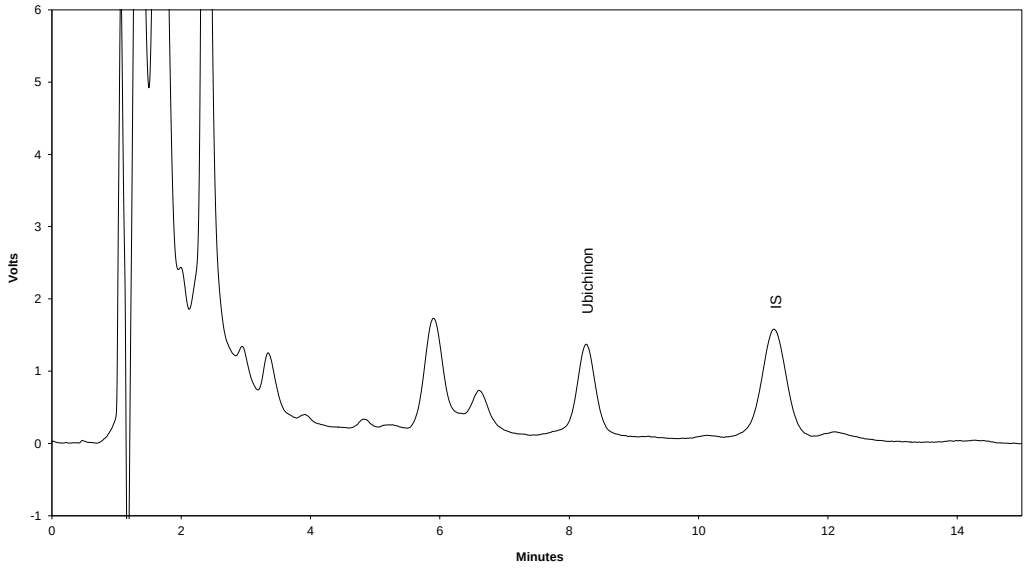
9. Calculation of analytical results

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 develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.
Mortensen S. A. et al: (1997) Molec.Aspects Med. 18:137-144

11. Validation data

Precision and reproducibility

Intra-Assay CV:	2.1 % (0.52 µg/ml)	[n = 6]
	0.6 % (1.23 µg/ml)	[n = 6]
Inter-Assay CV:	6.0 % (0.50 ng/ml)	[n = 6]
	6.2 % (1.17 µg/ml)	[n = 6]

Linearity

up to 20 µg/ml

Detection limit

0.02 µg/ml

Recovery

98.4 %

12. Disposal

The mobile phase (ELU), extraction solution (EXTR), internal standard (IS), and ethanol p.A. (ETHA) must be disposed as non-halogenated solvent. Please refer to the appropriate national guidelines.

13. Troubleshooting

Problem	Possible reason	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
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

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.