



CORTISOL AND CORTISONE IN SALIVA BY LC-MS/MS

REF LC91110

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.



INSTRUCTION FOR USE

IVD

For *in vitro* diagnostic use

CE



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INTENDED USE

In vitro diagnostic medical device for the determination and quantitative analysis of Cortisol and Cortisone in saliva as a monitoring of psychophysical stress and as an aid to the diagnosis of Cushing's disease. Manual method for professional use.

KIT CONTENT FOR 100 TESTS

CONTENT	DESCRIPTION	PACKAGING	WARNING
Reagent A	Internal Standard Solution	1 x 1 ml	Store at -25°C 
Reagent B	Clarifying Solution	1 x 2 ml	Store at $+2^{\circ}\text{C}$ 
Reagent C	Stabilization Solution	1 x 10 ml	Store at $+2^{\circ}\text{C}$ 
Multiparametric Calibrator in saliva		7 x 1 x 3 ml	(packed separately – Code LC91116 – see data sheet)
Reagent M1	Mobile Phase M1	2 x 500 ml	Store at $+2^{\circ}\text{C}$ 
Reagent M2	Mobile Phase M2	2 x 500 ml	Store at $+2^{\circ}\text{C}$ 

ACCESSORIES AND CONSUMABLES AVAILABLE SEPARATELY

CODE	DESCRIPTION	PACKAGING
LC91116	Calibrator for Cortisol and Cortisone in saliva	7 x 2 x 3 ml
LC91119	Control for Cortisol and Cortisone in saliva – Levels 1, 2 e 3	3 x 5 x 3 ml
SK91110	Starter kit for Cortisol and Cortisone in saliva	3 x 1 ml
LC91111	Mobile Phase M1	4 x 500 ml
LC91121	Mobile Phase M2	4 x 500 ml
LC91130	Deuterated Internal Standard Solution in saliva	1 x 1 ml
Z699675902	Poroshell 120-EC-18 C18 Analytical Column (50 x 2,1 mm - 1,9 µm)	1 Pc
L-30-210	Eppendorf test tubes by 1,5 ml with caps	1 x 1000 Pcs
Z51843550	Clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml	1 x 100 Pcs
Z51820717	Caps for clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml and for amber vials	1 x 100 Pcs

INSTRUMENTS

DESCRIPTION	WARNINGS
LC-MS triple quadrupole instrument (MRM Modality) Divert Valve at 6 ways ESI Interface (+) Binary Pump	Required
Operational Computer	Required
Autosampler	Required

MATRIX – COLLECTION AND STORAGE PROCEDURE

MATRIX	PROCEDURE
Saliva	The patient must be fasting and must not have carried out intense sporting activity for at least 2 hours before the sampling. 10 minutes before collection, you will have to rinse your mouth with cold water; note down the time of collection. The collection must take place with devices suitable for the collection of salivary samples such as Salivette Cortisol or similar. It is recommended that the collector has to be centrifuged immediately to improve sample stability. Saliva samples are stable up to 7 days if stored at $+15^{\circ}\text{C}$  $+25^{\circ}\text{C}$, 3 months at $+2^{\circ}\text{C}$  $+8^{\circ}\text{C}$ up to 1 year if stored at -25°C  -15°C.

REFERENCE VALUES

Hormones	Normal Range (ng/ml)
Cortisol	H 7 – 9 a.m.: 1 – 7,5 H 3 – 5 p.m.: < 4,01 H 11 – 12 p.m.: < 1
Cortisone	Morning: 3,7 – 15,3 Night: 0,54 – 4,7

ANALYTICAL PROCEDURE

STEP	DESCRIPTION																								
1	Sample Preparation																								
	In Eppendorf tubes dispense in sequence:																								
	<table><tr><td></td><td>Calibrator (µl)</td><td>Sample (µl)</td><td>Controls (µl)</td></tr><tr><td>Calibrator</td><td>500</td><td></td><td></td></tr><tr><td>Sample</td><td></td><td>500</td><td></td></tr><tr><td>Controls</td><td></td><td></td><td>500</td></tr><tr><td>Reagent A</td><td>10</td><td>10</td><td>10</td></tr><tr><td>Reagent B</td><td>20</td><td>20</td><td>20</td></tr></table>		Calibrator (µl)	Sample (µl)	Controls (µl)	Calibrator	500			Sample		500		Controls			500	Reagent A	10	10	10	Reagent B	20	20	20
		Calibrator (µl)	Sample (µl)	Controls (µl)																					
	Calibrator	500																							
	Sample		500																						
	Controls			500																					
	Reagent A	10	10	10																					
Reagent B	20	20	20																						
Vortex for 20 seconds.																									
2	Centrifuge at 14.000 rpm for 10 minutes.																								
3	Dispense in Eppendorf tubes 300 µl of supernatant and add 100 µl of <u>Reagent C.</u> Vortex for 20 seconds.																								
INJECTION	Inject 15 µl of the solution in the LC system.																								

GRADIENT

TIME (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.0	65	35	400
3.00	45	55	400
4.00	45	55	400
4.01	2	98	400
5.50	2	98	400
5.51	65	35	400
7.50	65	35	400

PARAMETERS

Parameters set on triple quadrupole

	ESI+ (SCIEX 4500)
CURTAIN GAS	25
COLLISION GAS	9
IONSPRAY VOLTAGE	5500 V
TEMPERATURE	600°C
ION SOURCE GAS 1	50
ION SOURCE GAS 1	60

FRAGMENTATION OF THE ANALYTE AND THE DEUTERATED INTERNAL STANDARD (IS)

ANALYTE	RT	FRAGMENTS		DEUTERATED IS USED FOR CALIBRATION	RT	FRAGMENTS	
		PARENT ION (Q1)	FRAGMENT ION (Q3)			PARENT ION (Q1)	FRAGMENT ION (Q3)
Cortisol - 1	2.9	363.1	121.0	Cortisol-D4	2.9	367.1	121.0
Cortisol - 2	2.9	363.1	115.1	Cortisol-D4	2.9	367.1	121.0
Cortisone - 1	2.6	361.2	163.0	Cortisol-D4	2.9	367.1	121.0
Cortisone - 2	2.6	361.2	121.0	Cortisol-D4	2.9	367.1	121.0

TECHNICAL FEATURES - PART I

ABBREVIATIONS	MEANING
C LLOQ	Concentration at low limit quantification
C1	Low concentration
C2	Medium concentration
C3	High concentration
Max C	The highest concentration

ANALYTE	CONCENTRATIONS USED TO CALCULATE REPRODUCIBILITY AND ACCURACY (ng/ml)				
	C LLOQ	C1	C2	C3	MAX C
Cortisolo	0,1	1	10	25	50
Cortisone	0,1	1	10	25	50

ANALYTE	ACCURACY INTRA SERIE (RELATIVE ERROR %)		ACCURACY INTER SERIE (RELATIVE ERROR %)	
	C1 (%)	C3 (%)	C1 (%)	C3 (%)
Cortisol	3,59	1,03	11,41	2,61
Cortisone	4,94	1,98	6,92	3,82

TECHNICAL FEATURES - PART II

ANALYTE	REPRODUCIBILITY INTRA SERIE (COEFFICIENT OF VARIATION %)			REPRODUCIBILITY INTER SERIE (COEFFICIENT OF VARIATION %)		
	C LLOQ %	C2 %	Max C %	C LLOQ %	C2 %	Max C %
Cortisol	5,10	4,76	4,22	10,40	6,44	6,15
Cortisone	3,71	5,51	3,93	8,56	8,87	5,78

TECHNICAL FEATURES - PART III

ANALYTE	LLOD (ng/ml)	LINEARITY (ng/ml)	COEFFICIENT OF CORRELATION R2 + DEV STD
Cortisol	0,03	0,1 – 500	0,9993 ± 0,0003
Cortisone	0,03	0,1 – 500	0,9989 ± 0,0009

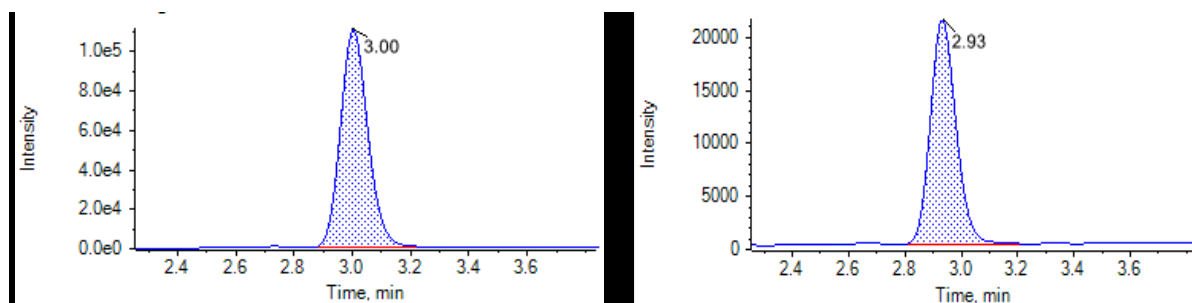
REFERENCE CHROMATOGRAMS

Fig. 1 Cortisol Calibrator at 50 ng/ml (left panel) and Internal Standard (right panel)

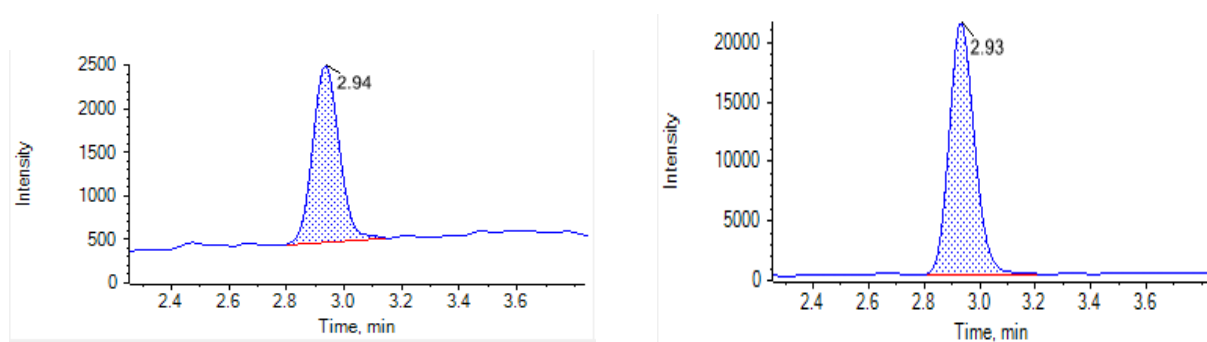


Fig. 2 Cortisol Control - Level 1 at 0.5 ng/ml (left panel) and Internal Standard (right panel)

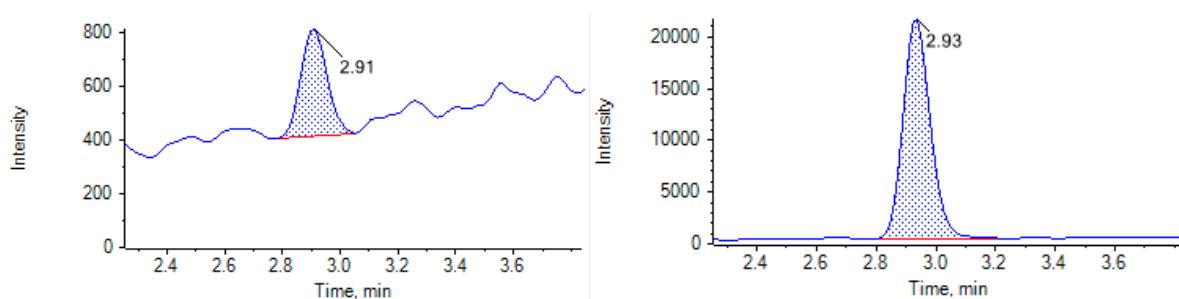


Fig. 3 Saliva Sample: Cortisol 0.2 ng/ml (left panel) and Internal Standard (right panel)

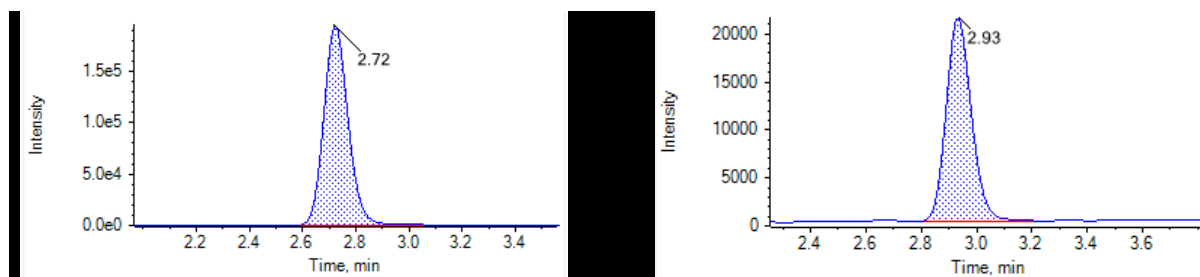


Fig. 4 Cortisone Calibrator at 50 ng/ml (left panel) and Internal Standard (right panel)

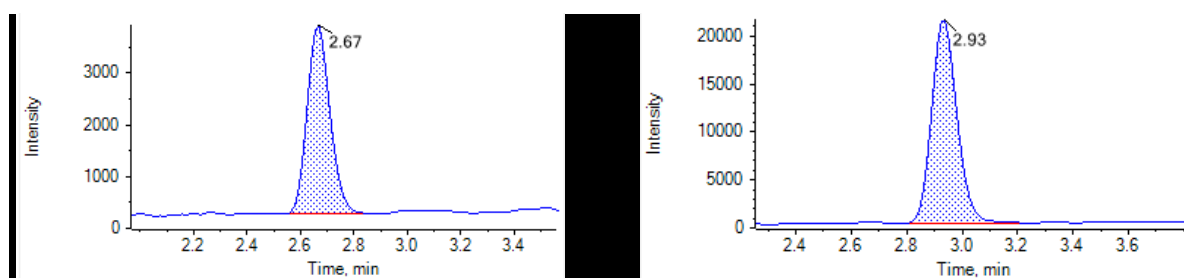


Fig. 5 Cortisone Control - Level I at 0.5 ng/ml (left panel) and Internal Standard (right panel)

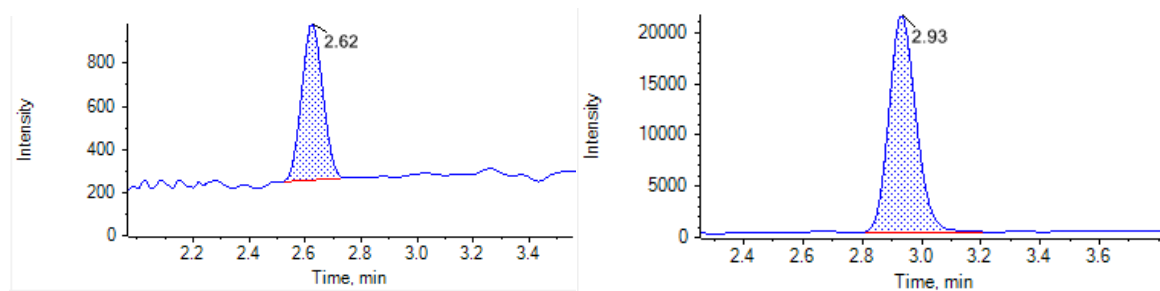


Fig. 6 Saliva Sample: Cortisone 0.2 ng/ml (left panel) and Internal Standard (right panel)

GENERAL WARNINGS

1. STORAGE AND SHELF LIFE

All the components are ready to use. They are stable for 3 years at  +2 °C except the

Reagent A (Internal Standard Solution) that must be stored at  -25 °C.

After the opening the shelf life of our Internal Standards is 6 months if stored at  -25 °C.

2. NEW COLUMN TREATMENT

Install the new analytical column Poroshell 120 EC C18 (50 x 2,1 mm - 1,9 µm) thermostated at 45°C. Disconnect the detector and flux a solution of Mobile Phase M1: Mobile Phase M2 (20:80 v/v) and set flow at 400 µl / minutes. Don't recycle the washing solutions.

3. COLUMN CONDITIONING

Conditionate the new analytical column Poroshell 120 EC C18 (50 x 2,1 mm - 1,9 µm) with Mobile Phases M1: M2 (20:80 v/v), set flow at 400 µl / minute for 10 minutes. Then flux a solution of Mobile Phase M1: Mobile Phase M2 (80:20 v/v) and set flow at 400 µl / minute for 5 minutes. Then flux a solution of Mobile Phase M1: Mobile Phase M2 (65:35 v/v) and set flow at 400 µl / minute for 10 minutes. In the end, make 3 injections of Mobile Phase before the analytical session. It's not possible to make analysis at recycling phase. If room temperature >20°C, store the mobile

phase  +2 °C from an analytical session to an another.

4. COLUMN CLEANING

Disconnect the detector. Wash the column with a solution of Mobile Phase M1: Mobile Phase M2 (80:20 v/v) and set flow at 400 µl / minute for 5 minutes and throw it. Then flux a solution of Mobile Phase M1: Mobile Phase M2 (10:90 v/v) and set flow at 400 µl / minute for 15 minutes. Store the column in a solution of Mobile Phase M1: M2 (10:90 v/v).

5. AUTOSAMPLER SYRINGE WASHING

Wash the syringe and the needle with Mobile Phase M2.

ABBREVIATIONS

ABBREVIATIONS	MEANING
Es. 5 x 1 x 1 ml	N° of levels x N° of vials x volume
e.g. packaging of the starter kit: 27 x 1 ml	N° di vial x volume Note: each vial contains one molecule (analyte or labelled IS)
IS	Internal Standard
Pc/pcs	Piece/Pieces
RT	Retention Time
STD DEV	Standard Deviation

BIBLIOGRAPHY

1. Antonelli G. *et al.* Salivary cortisol and cortisone by LC-MS/MS: Validation, reference intervals and diagnostic accuracy in Cushing's syndrome Clinica Chimica Acta 2015 Volume 451 Parte B
2. Ursula Turpeinen *et al.* Determination of salivary cortisol by liquid chromatography-tandem mass spectrometry Scandinavian Journal of Clinical & Laboratory Investigation Vol. 69, No. 5, September 2009, 592–597
3. José Gilberto Henriques Vieira *et al.* Determination of cortisol and cortisone in human saliva by a liquid chromatography-tandem mass spectrometry method Arq Bras Endocrinol Metab. 2014, 58/8

REFERENCE STANDARDS

Quality Assurance System compliant with regulations:

UNI CEI EN ISO 13485:2016,

UNI EN ISO 9001:2015

Any modification made to the Medical Device unless authorized by Eureka S.r.l. - Lab Division makes this declaration invalid.

We declare that the devices comply with the following **Technical Standards**:

UNI CEI EN ISO 14971:2012

UNI CEI EN ISO 15223-1:2017

UNI EN ISO 18113-2:2012

We declare that the device is sold in a non-sterile package.

IVD SYMBOLS

NUMBER	SYMBOL	DESCRIPTION
1		Manufacturer
2		Product Model Number / Designation or Part Number
3		Caution, consult accompanying documents
4		Product Lot Number
5		Use By (Expiry)
6		Consult Operating Instructions
7		Date of manufacture
8		Temperature limitation
9		For <i>in vitro</i> diagnostic use
10		European Union conformity
11		Packaging