Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it -www.listarfish.it

Useŕs Manual

Cortisol free in Saliva ELISA

IVD

CE

REF **DEM-DES6611**

Σ **96 Wells**

CONTENTS / INHALTSVERZEICHNIS

1 INTRODUCTION 2

2 PRINCIPLE 2

3 WARNINGS AND PRECAUTIONS 2

4 REAGENTS 3

5 SPECIMEN COLLECTION AND PREPARATION 4

6 ASSAY PROCEDURE 5

7 EXPECTED NORMAL VALUES 7

8 QUALITY CONTROL 7

9 PERFORMANCE CHARACTERISTICS 7

10 LIMITATIONS OF PROCEDURE 9

11 LEGAL ASPECTS 10

12 REFERENCES 10

SYMBOLS USED WITH DEMEDITEC ELISA´S 20

1 INTRODUCTION

1.1 Intended Use

An enzyme immunoassay for the quantitative in vitro diagnostic measurement of active free cortisol (hydrocortisone and hydroxycorticosterone) in saliva.

1.2 Summary and explanation

Cortisol is a corticosteroid hormone or glucocorticoid produced by the adrenal cortex, that is part of the adrenal gland (in the Zona fasciculata and the Zona reticularis of the adrenal cortex). It is usually referred to as the "stress hormone" as it is involved in response to stress. It increases blood pressure and blood sugar, and reduces immune responses.
The amount of Cortisol present in saliva undergoes diurnal variation. During the first 2 or 3 hours after typical wake-up time there is a distinct concentration peak-value. The position of this peak-value is strongly influenced by the average wake-up time during the past week. It is not as dependent on the actual wake-up time of the specific day of sample collection (if different from the average wake-up time of the past week). After this peak the Cortisol concentration declines until approximately midnight. The best time for sample collection to test for diseases such as Morbus Cushing (Cushing's Disease) is midnight [(1)]. Spontaneous increases in Cortisol concentration during the day may occur, commonly due to stress or food intake. Strenuous physical exercise can also result in increased Cortisol concentrations post-exercise. Exercise-induced increases in Cortisol concentration have been reported to even exceed the morning peak concentration. After several hours post-exercise the concentration should return to normal levels.
The diurnal pattern of Cortisol excretion is not present at birth (estimates of when it starts vary from two weeks to 9 months). Changed patterns of Cortisol levels have been observed in connection with abnormal ACTH levels, clinical depression, psychological stress, and various physiological stressors as hypoglycemia, illness, fever, trauma, surgery, fear, or pain.

2 PRINCIPLE

The **DEMEDITEC Cortisol free in Saliva ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal rabbit antibody directed against the cortisol molecule. Endogenous cortisol of a patient sample competes with a cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of cortisol in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.

10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec Diagnostics GmbH.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12x8 (break apart) strips, 96 wells;
   Wells coated with an anti-cortisol antibody (polyclonal).
2. **CAL 0 Calibrator 0,** 1 vial, 2.0 ml, ready to use
3. **CAL 1-5 Calibrator (Calibrator 1-5),** 5 vials, 0.5 ml each, ready to use;
   Concentrations: 0.1 - 0.4 - 1.7 – 7.0 – 30 ng/ml,
   Conversion factors: 1 ng/ml = 2.762 nmol/l = 0.1 µg/dl
4. **CONTROL 1-2 Control low** / **Control high**, 2 vials, 0.5 ml each, ready to use;
   For control values and ranges please refer to QC-Datasheet.
5. **ENZ CONJ Enzyme Conjugate**, 1 vial, 7.0 ml, ready to use;
   Cortisol conjugated to horseradish peroxidase;
6. **SUB TMB Substrate Solution**, 1 vial, 22 ml, ready to use;
   Tetramethylbenzidine (TMB).
7. **STOP SOLN Stop Solution**, 1 vial, 7.0 ml, ready to use;
   contains 2 N acidic solution.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (**10X** concentrated);
   see „Preparation of Reagents“.

All reagents contain azide-free and mercury-free preservatives.

Note: Additional *Calibrator 0* for sample dilution is available upon request.

4.2 Material required but not provided

- Microcentrifuge
- A microtiter plate calibrated reader (450±10 nm)
- Microplate mixer operating at about 600-900 rpm, optionally
- Vortex mixer
- Calibrated variable precision micropipettes (50 µl, 100 µl, 200 µl).
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage conditions

When stored at 2 °C to 8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.4 Reagents preparations

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

Wash Solution
Add deionized water to the 10X concentrated *Wash Solution*.
Dilute 50 ml of concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for 3 months at room temperature.

4.5 Disposal of the kits

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, DEMEDITEC have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give falsely elevated concentration values. In case of visible blood contamination the patient should discard the sample, rinse the collection device with water, also rinse the mouth with (preferably) cold water, wait for 10 minutes and take a new sample. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva sample.

5.1 Specimen Collection

For the correct collection of saliva we are recommending to only use appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or Salivettes for sampling; this in most cases will result in significant interferences. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper. Please contact Demeditec Diagnostics for more details.

As the Cortisol secretion in saliva as well in serum shows an obvious secretion pattern throughout the day it is important to care for a proper timing of the sampling. The morning peak normally appears during the first 2 hours after the average wake-up time. But also during the day there might be smaller peaks in the Cortisol secretion. Therefore we recommend taking 5 separate samples within a period of 2 hours (multiple sampling) directly after the usual wake-up time. As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem it is allowed to eat small amount of vegetarian food directly after collecting one sample. After eating such a small amount the patient should clean his mouth by washing it with water. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Saliva flow may be stimulated by drinking water. This is allowed and even recommended before and during the collection period. Drinking of water is not allowed during the last 5 minutes before taking a saliva sample. It is important to know that the timing of the morning peak is not related to the absolute time or the day light. It is just related to the wake-up habits of the patient. In order to catch the peak we recommend collecting the samples at approximately 1 min, 30 min, 60 min, 90 min, and 120 minutes after the usual weak-up time of the last 10 days. The typical timing for a morning collection period would be as follows. Wake-up at 6:00 AM, 1st sample at 6:01 AM, drinking water and brushing teeth, followed by samples at 6:30 AM, 7:00 AM, 7:30 AM, and 8:00 AM, followed by breakfast at maybe 8:15 AM. Modest variation in the collection timing will not be critical, and the collection time-frame can be extended up to 3 hours. Special care has to be taken in case the patient recently has done trip over several time zones.

The collection for the evening sample (e.g. midnight Cortisol for the detection of Morbus Cushing) has to be done during the late evening (at best between 10 and 12 PM). Also in this case we recommend collecting 5 samples in intervals of at least 30 minutes. If only 5 sampling devices are available for the collection of a day profile, sampling also can be done as follows. 30 min, 60 min, and 90 minutes after the usual wake-up time for the morning value; followed by 2 samples in the late evening collected during the last hour before going to bed.

5.2 Specimen Storage and Preparation

Saliva samples in general are stable at ambient temperature for several days. Therefore mailing of such samples by ordinary mail without cooling will not create a problem. Storage at 4°C can be done for a period of up to one month. Whenever possible samples preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully. Then the samples have to be centrifuged for 5 to 10 minutes. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slight reddish tinge it should be discarded. Otherwise the concentration value most probably will be falsely elevated. Due to the episodic variations of the steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples has to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture. If the shape of the morning peak has to be determined all 5 morning samples have to be tested separately.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest calibrator, the specimens can be diluted with *Calibrator 0* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µl saliva + 90 µl *Calibrator 0* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a) + 90 µl *Calibrator 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.

- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.

6.2 Assay procedure

Each run must include a standard curve.

1. Secure the desired number of coated strips in the frame holder.
2. Dispense **50 µl** of each **Calibrator, Control and sample** with new disposable tips into appropriate wells.
3. Dispense **50 µl** of **Enzyme Conjugate** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature. Shaking on a horizontal shaker during incubation is not necessary, but it improves the sensitivity of the test slightly.
5. Briskly empty the contents of the wells by aspiration or by decanting.
   Rinse the wells 4 times with diluted Wash Solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **200 µl** of **Substrate Solution** to each well.
7. Incubate for **30 minutes** in the dark at room temperature.
8. Stop the enzymatic reaction by adding **50 µl** of **Stop Solution** to each well.
9. Determine the absorbance of each well at **450±10 nm**.
   It is recommended that the wells be read within 15 minutes.

6.3 Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each calibrator against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of typical calibrator curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Calibrator		**Optical Units (450 nm)**
Calibrator 0	0.0 ng/ml	2.347
Calibrator 1	0.1 ng/ml	2.011
Calibrator 2	0.4 ng/ml	1.512
Calibrator 3	1.7 ng/ml	0.848
Calibrator 4	7.0 ng/ml	0.462
Calibrator 5	30 ng/ml	0.187

7 EXPECTED NORMAL VALUES

In order to determine the normal range of Cortisol free in Saliva samples from adult male and female apparently healthy subjects, were collected and analyzed using the DEMEDITEC Cortisol free in Saliva ELISA kit. The following range was calculated from this study.

Time of day	5-95% percentile (ng/ml)	n
Morning	1 – 11.3	234
Midday	0.3 – 5.7	427
Afternoon	0.3 – 3.3	129
Evening	0.2 – 2.7	419
Midnight	< 1.0	26

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.
Since Cortisol levels show diurnal cycles, we recommend to always collecting a series of samples in the morning and another one in the evening. The difference between morning and evening is the important parameter. Furthermore, we recommend that each laboratory determines its own range for the population tested.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DEMEDITEC directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Analytical Sensitivity

The analytical sensitivity of the DEMEDITEC ELISA was calculated by subtracting 2 standard deviations from the mean of twenty (20) replicate analyses of *Calibrator 0*. The analytical sensitivity of the assay is 0.024 ng/ml.

9.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity.

Steroids	% Crossreactivity
Testosterone	< 0.1%
Corticosterone	5.2%
Cortisone	0.2%
11-Deoxycorticosterone	0.4%
11-Deoxycortisol	10.4%
Dexamethasone	< 0.1%
Estriol	< 0.1%
Estrone	< 0.1%
Prednisolone	63.4%
Prednisone	< 0.1%
Progesterone	< 0.1%
Danazole	< 0.1%
Pregnenolone	< 0.1%
Estradiol	< 0.1%

9.3 Assay Dynamic Range

The range of the assay is between 0.1 – 30 ng/ml.

9.4 Reproducibility

9.4.1 Intra-Assay

The intra-assay variation was determined by replicate measurements of 2 saliva samples within one run using the DEMEDITEC Cortisol free in Saliva ELISA. The within-assay variability is shown below:

	Sample 1	Sample 2
Mean (ng/ml)	6.74	2.701
SD (ng/ml)	0.39	0.102
CV (%)	5.8	3.8
n =	20	20

9.4.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of 2 saliva samples in 10 different runs using the DEMEDITEC Cortisol free in Saliva ELISA. The inter-assay variability is shown below:

	Sample 1	Sample 2
Mean (ng/ml)	1.15	8.22
SD (ng/ml)	0.071	0.53
CV (%)	6.2	6.4
n =	10	10

9.5 Recovery

Recovery of the DEMEDITEC ELISA was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Saliva	Spiking	Observed (0)	Expected (E)	O/E %
1	-	0,54	-	-
	2 ng/mL	2,78	2,54	109%
	4 ng/mL	4,11	4,54	91%
	6 ng/mL	5,75	6,54	88%
2	-	1,1	-	-
	2 ng/mL	3,62	3,10	116%
	4 ng/mL	5,57	5,10	109%
	6 ng/mL	7,52	7,10	106%
3	-	4,48	-	-
	2 ng/mL	6,28	6,48	97%
	4 ng/mL	8,78	8,48	104%
	6 ng/mL	9,95	10,48	95%

9.6 Linearity

Three saliva samples containing different amounts of analyte were serially diluted with Calibrator 0 and assayed with the DEMEDITEC ELISA. The percentage recovery was calculated by comparing the expected and observed values for cortisol.

Saliva	Dilution	Observed (0)	Expected (E)	O/E %
1	1 in 1	6,46	-	-
	1 in 2	3,26	3,23	101%
	1 in 4	1,58	1,62	98%
	1 in 8	0,78	0,81	96%
2	1 in 1	7,35	-	-
	1 in 2	4,32	3,68	117%
	1 in 4	2,13	1,84	116%
	1 in 8	0,98	0,92	106%
3	1 in 1	18,38	-	-
	1 in 2	9,68	9,19	105%
	1 in 4	5,15	4,60	112%
	1 in 8	2,45	2,30	106%

10 LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 High-Dose-Hook Effect

No hook effect was observed in this test.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Cortisol in a sample.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DEMEDITEC.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

1. Authors: LK Nieman, BMK Biller, JW Findling, J Newell-Price, VM Montori
   Title: The diagnosis of Cushing's syndrome: An Endocrine Society Clinical Practice Guideline
   Published in: J. Clin. Endocrin. & Metabol. **2008**, Vol.93, No.5, pages 1526-1540
2. Kirchbaum C., Hellhammer DH. (**1989**): Salivary cortisol in psychobiological Research:
   An overview, Neuropsychobiology, 22, 150-169
3. Kirchbaum C, Hellhammer Dh. (**1994**): Salivary cortisol in psychoneuroendocrine Research: Recent developments and applications, Psychoneuroendocrinology, 19, pp 313-333
4. Vining RF, et al. (**1983**), Salivary cortisol: a better measure of adrenal cortical function than serum cortisol, Ann Clin Biochem, 20, 329-335
5. Authors: E.W. Harville, D.A. Savitz, N. Dole, A.H. Herring, J.M. Thorp, K.C. Light
   Title: Patterns of salivary Cortisol secretion in pregnancy and implications for assessment protocols.
   Published in: Biol. Psychol **2007**, Vol.74, pages 85-91
6. Authors: AH Thomson, MC Devers, AM Wallace, D. Grant, K. Cambell, JMC Connell
   Title: Variability in hydrocortisone plasma and saliva pharmacokinetics following intravenous and oral administration to patients with adrenal insufficiency.
   Published in: Clinical Endocrinology **2007**, Vol.66, pages 789-796
7. Authors: RJ Custodio, CE Martinelli, SLS Milani, AL Simoes, M de Castro, AC Moreira
   Title: The emerge of the Cortisol circadian rhythm in monozygotic and dizygotic twin infants: the twin-pair synchrony.
   Published in: Clinical Endocrinology **2007**, Vol 66, pages 192-197

8. Authors: MD Garcia-Prieto, FJ Tébart, F. Nicolàs, E. Larqué, M. Garaulet
Title: Cortisol secretary pattern and glucocorticoid feedback sensitivity in women from a Mediterranean area: Relationship with anthropometric characteristics, dietary intake and plasma fatty acid profile.
Published in: Clinical Endocrinology **2007**, Vol 66, pages 185-191
9. Authors: S. Izawa, N. Sugaya, N. Ogawa, Y. Nagano, M. Nakano, E. Nakase, S. Nomura
Title: Episodic stress associated with writing a graduation thesis and free Cortisol secretion after awakening.
Published in: International Journal of Psychophysiology **2007**, Vol 64, pages 141-145
10. Authors: DS Jessop, JM Turner-Cobb
Title: Measurement and meaning of salivary Cortisol: A focus on health and disease in children
Published in: Stress **2007**, Preprint, accepted 25 March 2007, pages 1-14
11. Authors: DB Fekedulegn, ME Andrew, CM Burchfiel, JM Violanti, DB Miller
Title: Area under the curve and other summary indicators of repeated waking Cortisol measurements.
Published in: Psychosomatic Medicine **2007**, Vol. 69, pages 651-659
12. Authors: I. Wilhelm, J. Born, B.M. Kudielka, W. Schlotz, S. Wüst
Title: Is the Cortisol awakening rise a response to awakening?
Published in: Psychoneuroendocrinology **2007** Vol.32. pages 358-366
13. Authors: B.M. Kudielka, S. Bellingrath, D.H. Hellhammer
Title: Further support for higher salivary Cortisol levels in “morning” compared to “evening” persons.
Published in: Journal of Psychosomatic Research **2007** Vol.62, pages 595-596
14. Authors: L.D. Dorn, J.F. Lucke, T.L. Loucks, S.L. Berga
Title: Salivary Cortisol reflects serum Cortisol: Analysis of circadian profiles.
Published in: Ann Clin Biochem **2007** Vol. 44, pages 281-284
15. Authors: R.L. Matchock, L.D. Dorn, E.J. Susman
Title: Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty
Published in: Chronobiol Int. **2007**, Vol. 24(5), pages 969-90

SYMBOLS USED WITH DEMEDITEC ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Estándar 0	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante

Symbol	Portugues	Dansk	Svenska	Ελληνικά
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
RUO				
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..
Microtiterwells	Alvéolos de microtitulação	Mikrotiterbrønde	Brunnar i Mikrotiterplatta	Πηγαδάκια Μικροτιτλοδοτήσεως
Antiserum	Anti-soro	Antiserum	Antiserum	Αντιορός
Enzyme Conjugate	Conjugado enzimático	Enzymkonjugat	Enzymkonjugat	Συζευγμένο ενζυμο
Enzyme Complex	Complexo enzimático	Enzymkompleks	Enzymkomplex	Σύμπλοκο ενζύμου
Substrate Solution	Solução de substrato	Substratopløsning	Substratlösning	Διάλυμα υποστρώματος
Stop Solution	Solução de paragem	Stopopløsning	Stopp lösning	Διάλυμα τερματισμού
Zero Standard	Padrão zero	Standard 0	Standard 0	Πρότυπο Μηδέν
Standard	Calibrador	Standard	Standard	Πρότυπα
Control	Controlo	Kontrol	Kontroll	Έλεγχος
Assay Buffer	Tampão de teste	Assay buffer	Assay Buffer	Ρυθμιστικό Διάλυμα Εξέτασης
Wash Solution	Solução de lavagem	Vaskebuffer	Tvätt lösning	Διάλυμα πλύσεως
Sample Diluent				
Conjugate Diluent				