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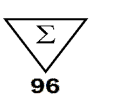
Instructions for use

Cortisol Saliva ELISA Free

REF SA E-6000



IVD

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Cortisol Saliva ELISA [Free]

Introduction

Intended Use

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

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.

PRINCIPLE

The **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.

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. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
19. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
20. Safety Data Sheets for this product are available upon request directly from Diagnostics GmbH. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

REAGENTS

Reagents provided

96 **SA E-6031 Microtiterwells**

12x8 (break apart) strips, 96 wells; Wells coated with a anti-cortisol antibody (polyclonal).

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**SA E-6001**	Standard 0	0 ng/ml	3 ml
STANDARD B	**SA E-6002**	Standard 1	0.1 ng/ml	1 ml
STANDARD C	**SA E-6003**	Standard 2	0.4 ng/ml	1 ml
STANDARD D	**SA E-6004**	Standard 3	1.7 ng/ml	1 ml
STANDARD E	**SA E-6005**	Standard 4	7 ng/ml	1 ml
STANDARD F	**SA E-6006**	Standard 5	30 ng/ml	1 ml

Conversion factor: 1 ng/mL = 2.76 nmol/L; contain 0.003% Proclin as a preservative

CONTROL 1 + CONTROL 2 **SA E-6051 + SA E-6052 Control low / Control high**

2 vials, 1 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet; ready to use

CONJUGATE **SA E-6040 Enzyme Conjugate**

1 vial, 7 mL, ready to use; Cortisol conjugated to horseradish peroxidase; ready to use

SUBSTRATE **AR E-0055 Substrate Solution** 1

vial, 22 ml each, ready to use; contains tetramethylbenzidine (TMB)

STOP-SOLN **AR E-0080 Stop Solution**

1 vial, 7 ml, ready to use

WASH-CONC 10x **AR E-0030 Wash Solution**

1 vial, 50 ml (10X concentrated); see „Preparation of Reagents".

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

Material required but not provided

- Microcentrifuge
- A microtiter plate calibrated reader (450±10 nm)
- Microplate mixer operating at about 600 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

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.

Reagents preparations

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.

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.

Damaged test kits

In case of any severe damage of the test kit or components, 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.

SPECIMEN Collection and Preparation
Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

If there is visible blood contamination the patient specimen, it should be discarded, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Saliva samples should be collected only using special saliva sampling devices (vial and straw),
e.g. **Sali Set** (catalogue no. SA D-6100, 100 pieces).

Due to the cyclic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling. In order to avoid arbitrary results we recommend that 5 samples always be taken within a period of 2 – 3 hours (*multiple sampling*) preferably before a meal.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

Specimen Storage and Preparation

The saliva samples may be stored at 2 °C to 8 °C up to one week, and should be frozen at –20 °C for longer periods; repeated thawing and freezing is no problem.

Each sample has to be frozen, thawed, and centrifuged at least once 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 (at 2000 - 3000 x g).
Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the 5 single samples in a separate sampling device and perform the testing from this mixture.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 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 *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a) + 90 µl *Standard 0* (mix thoroughly).

ASSAY PROCEDURE

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.

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 ***Standard, Control*** **and samples** with new disposable tips into appropriate wells.
3.	Dispense **50 µL** ***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 shake out the contents of the wells. Rinse the wells 3 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** 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.

Calculation of results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard 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 standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

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	2.347
Calibrator 1	2.011
Calibrator 2	1.512
Calibrator 3	0.848
Calibrator 4	0.462
Calibrator 5	0.187

EXPECTED NORMAL VALUES

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

Time of day	Range (5-95%)	n
Morning	1 – 11.3	234
Midday	0.3 – 5.7	427
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.

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 directly.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the 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.

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%

Assay Dynamic Range

The range of the assay is between 0 – 30 ng/ml.

Reproducibility

Intra-Assay

The intra-assay variation was determined by replicate measurements of 2 saliva samples within one run using the 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

Inter-Assay

The inter-assay variation was determined by duplicate measurements of 2 saliva samples in 10 different runs using the 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

Recovery

Recovery of the ELISA was determined by adding increasing amounts of the analyte to 4 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%

Linearity

Five saliva samples containing different amounts of analyte were serially diluted with Standard 0 and assayed with the ELISA.
The percentage recovery was calculated by comparing the expected and measured 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%

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.

High-Dose-Hook Effect

No hook effect was observed in this test

Drug Interferences

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

LEGAL ASPECTS

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.

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.

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.

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!