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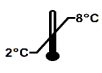
Instructions for use

Corticosterone ELISA

REF **MS E-5400R**



RUO

For research use only – Not for use in diagnostic procedures

Corticosterone ELISA

1 INTRODUCTION

1.1 Intended Use

The **Corticosterone ELISA** is an enzyme immunoassay for the quantitative measurement of Corticosterone in serum or plasma (lithium heparin or citrate plasma).

2 PRINCIPLE OF THE TEST

The Corticosterone ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the **principle of competitive binding**.
The microtiter wells are coated with a polyclonal antibody directed towards a unique antigenic site of the corticosterone molecule. During the first incubation, the corticosterone in the added sample competes for the binding to the coated antibody with the added conjugate molecule, which is a Corticosterone molecule conjugated to a horse-radish peroxidase. After incubation the unbound conjugate and sample are washed off. Following the addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of corticosterone in the sample. After incubation of substrate solution, the colorimetric reaction is abruptly stopped by adding stop solution.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. 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.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. 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.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flus immediately with water.
19. 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.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

4 REAGENTS

4.1 Reagents provided

MS E-5431 96 **Microtiterwells**

Content: 12x8 (break apart) strips, 96 wells; Wells coated with an anti-Corticosterone antibody (polyclonal).

Standards and **Controls** - ready to use

Cat. no.	Component	Standard	Concentration nmol/l	Volume / vial
MS E-5401	CAL 0	Standard A	0	1 ml
MS E-5402	CAL 1	Standard B	5	1 ml
MS E-5403	CAL 2	Standard C	15	1 ml
MS E-5404	CAL 3	Standard D	30	1 ml
MS E-5405	CAL 4	Standard E	60	1 ml
MS E-5406	CAL 5	Standard F	120	1 ml
MS E-5407	CAL 6	Standard G	240	1 ml
MS E-5151	CONTROL 1	Control 1	For control values and ranges please refer to vial label or QC-Datasheet.	1 ml
MS E-5152	CONTROL 2	Control 2		1 ml

Conversion: 1 nmol/l = 34.646 ng/dl
= 0.34646 ng/ml

The standards are calibrated against the following reference material: Cerilliant, code C-117
Contain non-mercury preservative.

MS E-5440 CONJUGATE-CONC 250x **Enzyme Conjugate** - **250X** concentrate

Content: Corticosterone conjugated to horseradish peroxidase; see „Reagent Preparation".

Volume: 1 x 150 µl

MS E-5461 CONJUGATE-DIL **Conjugate Diluent** - ready to use

Content: Contains non-mercury preservative.

Volume: 1 x 25 ml

SA E-0055 SUBSTRATE **Substrate Solution** - ready to use

Content: Tetramethylbenzidine (TMB)

Volume: 1 x 25 ml

FR E-0080 STOP-SOLN **Stop Solution** - ready to use

Content: contains 0.5 M H_2SO_4.

Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 14 ml

Hazards identification:

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

FR E-0030 WASH- CONC 40x **Wash Solution** - 40X concentrated

Volume: 1 x 30 ml
see „ Reagent Preparation "

Note: Additional *Standard A* for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm – 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2°C to 8°C unopened reagents will retain reactivity 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.
Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml distilled water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate
Dilute *Enzyme Conjugate* concentrate 1 + 249 in *Conjugate Diluent*.
This solution should be prepared freshly.

If the whole plate is used, dilute **100 µl** *Enzyme Conjugate* with **24.9 ml** *Conjugate Diluent*.
If the whole plate is not used at once prepare only the required quantity of Enzyme Conjugate.

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any damage to the test kit or components, the manufacturer must be informed in writing, at the latest one week after receiving the kit. 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 of according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (lithium heparin or citrate plasma) can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter "*Interfering Substances*".

5.1 Specimen Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 2 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time (up to 15 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

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

6.2 Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **20 µl** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3.	Dispense **200 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.
5.	Briskly shake out the contents of the wells. Rinse the wells **3 times** with **400 µl** diluted *Wash Solution* per well, if a plate washer is used – or – rinse the wells **3 times** with **300 µl** diluted *Wash Solution* per well for manual washing. 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 **100 µl** of ***Substrate Solution*** to each well.
7.	Incubate for **15 minutes** at room temperature.
8.	Stop the enzymatic reaction by adding **50 µl** of ***Stop Solution*** to each well.
9.	Determine the absorbance (OD) of the solution in each well at **450 nm (reading) and at 620 - 630 nm (background subtraction, recommended)** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi logarithmic graph paper, 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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 or reported as > 240 nmol/l. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

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

Standard	Optical Units (450 nm)
Standard A (0 nmol/l)	2.31
Standard B (5 nmol/l)	1.69
Standard C (15 nmol/l)	1.35
Standard D (30 nmol/l)	1.10
Standard E (60 nmol/l)	0.87
Standard F (120 nmol/l)	0.63
Standard G (240 nmol/l)	0.48

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy subjects, using the Corticosterone ELISA the following data were observed:

Population	n	Mean (nmol/l)	Median (nmol/l)	5^{th} - 95^{th} Percentile (nmol/l)	2.5^{th} - 97.5^{th} Percentile (nmol/l)	Range (min. - max.) (nmol/l)
Males	62	14.4	11.7	5.0 - 32.7	3.6 - 36.9	2.78 - 52.0
Females	64	11.7	9.4	3.0 - 29.6	2.1 - 41.7	1.6 - 50.0
Total	126	13.0	10.3	3.4 - 32.5	2.8 - 40.8	1.6 - 52.0

The values in **ng/dl** were calculated by multiplying the measured values (in nmol/l) by 34.646 as described in chapter 4.1.

Population	n	Mean (ng/dl)	Median (ng/dl)	5^{th} - 95^{th} Percentile (ng/dl)	2.5^{th} - 97.5^{th} Percentile (ng/dl)	Range (min. - max.) (ng/dl)
Males	62	494.9	402.0	173.7 - 1128.3	123.2 - 1272.6	95.5 - 1791.4
Females	64	401.5	322.2	104.9 - 1019.6	71.6 - 1436.9	55.1 - 1724.2
Total	126	447.4	355.0	117.1 - 1118.3	96.5 - 1405.7	55.1 - 1791.4

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard 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 sample 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 the manufacturer directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 1.68 – 240.0 nmol/l.

9.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Component	Cross-reactivity
Corticosterone	100 %
Progesterone	7.4 %
Deoxycorticosterone	3.4 %
11-Dehydrocorticosterone	1.6 %
Cortisol	0.3 %
Pregnenolone	0.3 %
Other steroids	<0.1 %

9.3 Sensitivity

The analytical sensitivity of the Corticosterone ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the *Standard A* and was found to be 0.589 nmol/l.

The Limit of Blank (LoB) is 0.527 nmol/l.
The Limit of Detection (LoD) is 1.680 nmol/l.
The Limit of Quantification (LoQ) is 4.462 nmol/l.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability was determined by measuring each sample 10 times per run (n = 10):

Sample	n	Mean (nmol/l)	CV (%)
1	10	6.8	7.7
2	10	62.3	3.8
3	10	122.5	3.3
4	10	160.0	5.8

9.4.2 Inter Assay

The between assay variability was determine by measuring each sample 10 times per run for 3 days (n = 30):

Sample	n	Mean (nmol/l)	CV (%)
1	30	7.0	10.8
2	30	63.0	6.1
3	30	115.6	6.4
4	30	153.9	5.0

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 3 different kit lots (n = 18):

Sample	n	Mean (nmol/l)	CV (%)
1	18	7.2	3.4
2	18	55.6	13.7
3	18	61.7	1.3
4	18	159.4	4.3

9.5 Recovery

Samples have been spiked by adding corticosterone solutions with known concentrations.
The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (nmol/l)		48.2	66.8	115.0	118.5
Average Recovery (%)		112.1	108.6	109.2	107.9
Range of Recovery (%)	from	110.1	107.3	103.6	99.0
	to	113.8	110.5	114.2	114.9

9.6 Linearity

Samples were measured undiluted and in serial dilutions with Standard A. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (nmol/l)		49.4	117.3	147.4	238.9
Average Recovery (%)		101.1	104.6	97.4	106.0
Range of Recovery (%)	from	96.2	100.6	91.8	95.0
	to	108.9	111.3	100.6	114.9

10 LIMITATIONS OF USE

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 Interfering Substances

Haemoglobin (up to 1 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 7.5 mg/ml) have no influence on the assay results.

10.2 Drug Interferences

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

10.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

11 REFERENCES/LITERATURE

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6. Rød A, et al, Scientific Reports, 2017, 7 (6748)

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!