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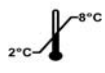
Instructions for use

17-OH-Progesterone ELISA

REF FR E-2800R



RUO

For research use only – Not for use in diagnostic procedures

17-OH-Progesterone ELISA

1. INTRODUCTION

1.1 Intended Use

Enzyme immunoassay for the determination of 17-OH-progesterone in human serum and plasma (EDTA).

2. PRINCIPLE

The assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

3. WARNINGS AND PRECAUTIONS

1. This kit is for research 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. All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
4. The microplate contains break apart 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 coloured. 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 (18 - 25 °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 microtiterplate 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. It may cause skin irritation and burns.
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 please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided

FR E-2831	96	**Microtiterplate**
Content:	12x8 (break apart) strips, 96 wells; Wells coated with an anti-17-OH-progesterone antibody (polyclonal).	

Standards and **Controls** - Ready to use

Cat. no.	Component	Concentration	Volume / Vial
FR E-2801	STANDARD A	0 ng/ml	1 ml
FR E-2802	STANDARD B	0.1 ng/ml	0.5 ml
FR E-2803	STANDARD C	0.4 ng/ml	0.5 ml
FR E-2804	STANDARD D	1.6 ng/ml	0.5 ml
FR E-2805	STANDARD E	6.5 ng/ml	0.5 ml
FR E-2806	STANDARD F	25 ng/ml	0.5 ml
FR E-2851*	CONTROL 1	For control values and ranges please refer to QC-Report!	0.5 ml
FR E-2852*	CONTROL 2		0.5 ml

* containing 17-OH Progesterone in serum.

FR E-2840 CONJUGATE **Enzyme Conjugate** - Ready to use

Content: 17-OH-progesterone conjugated to horseradish peroxidase

Volume: 1 x 11 ml

AR E-0055 SUBSTRATE **Substrate Solution** - Ready to use

Content: Tetramethylbenzidine (TMB)

Volume: 1 x 22 ml

Hazards identification:

H360D May damage the unborn child.

AR E-0080 STOP-SOLN **Stop Solution** - Ready to use

Content: contains 2 N acidic solution.
Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 7 ml

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

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

Volume: 1 x 50 ml

see "Reagent Preparation"

4.2 Material required but not provided

- Microcentrifuge
- A calibrated microtiter plate reader (450±10 nm)
- Microplate mixer operating at about 600 rpm (optional)
- Vortex mixer
- Calibrated variable precision micropipettes (25 µl, 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 - 8 °C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 - 8 °C. After first opening the reagents are stable for 30 days if used and stored properly.
Microtiter wells must be stored at 2 - 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 (18 - 25 °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 12 weeks 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 Safety Data Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, the manufacturer has to be informed in writing within 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

For determination of 17-OH-Progesterone serum or plasma (EDTA) can be used. The procedure calls for 25 µl sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain 17-OH-Progesterone concentrations higher than the highest **standard** (25 ng/ml) should be diluted with **Standard A** before assay. The additional dilution step has to be taken into account for the calculation of the results. Do not use grossly haemolytic, icteric or grossly lipaemic specimens.

6. ASSAY PROCEDURE

6.1 General remarks

- All reagents and specimens must be allowed to come to room temperature (18 -25°C) 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 **25 µl** of each **Standard**, **Control** and **samples** with new disposable tips into appropriate wells
3.	Dispense **100 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 slightly improves the sensitivity of the test.
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 standards, controls and 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 with Standard A. For the calculation of the concentrations this dilution factor has to be taken into account.

Conversion to SI units:
17-OH-Progesterone (ng/ml) x 3.03 = nmol/l

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	OD (450 nm)
Standard A 0.0 ng/ml	3.025
Standard B 0.1 ng/ml	2.653
Standard C 0.4 ng/ml	2.308
Standard D 1.6 ng/ml	1.638
Standard E 6.5 ng/ml	0.904
Standard F 25 ng/ml	0.443

7. EXPECTED NORMAL VALUES

Apparently healthy subjects show the following values of 17-OH-Progesterone:

Children	3 -14 years	0.05 - 2.0 ng/ml
Reproductive aged women	Follicular phase:	0.1 - 1.0 ng/ml
	Luteal phase:	0.6 - 2.5 ng/ml
	Ovulation:	0.3 - 1.5 ng/ml
	Post ACTH:	< 3.2 ng/ml
	Third trimester:	2.0 - 12 ng/ml
	Postmenopausal women:	0.13 - 0.6 ng/ml
Normal men		0.5 - 3 ng/ml

It is strongly recommended that each laboratory establishes its own range of normal values.

8. QUALITY CONTROL

Good laboratory practice requires to run controls 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-Report 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 Analytical Sensitivity

The analytical sensitivity of the *17-OH Progesterone ELISA* was calculated by subtracting 2 standard deviations from the mean of twenty (20) replicate analyses of Standard A. The analytical sensitivity of the assay is 0.0014 ng/ml.

9.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity.

Substance	% Cross-reactivity
Androstendione	< 0.1 %
Testosterone	< 0.1 %
Cortisol	0.05 %
11-Desoxycortisol	2.71 %
Cortisone	0.19 %
Corticosterone	< 0.1 %
11-Deoxycorticosterone	0.26 %
Progesterone	2.9 %
Estradiol	< 0.1 %
Estriol	< 0.1 %
Estrone	< 0.1 %
Pregnenolone	0.17 %
Prednisolone	< 0.1 %
Prednisone	0.11 %
DHEA	< 0.1 %
DHEA-S	< 0.1 %
Danazol	< 0.1 %
Dexamethasone	< 0.1 %

9.3 Assay Dynamic Range

The range of the assay is between 0.1 - 25 ng/ml.

9.4 Reproducibility

9.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 3 serum samples within one run using the *17-OH Progesterone ELISA*. The intra-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	0.52	2.29	5.86
SD (ng/ml)	0.05	0.16	0.38
CV (%)	8.9	6.9	6.6
n =	20	20	20

9.4.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of 3 serum samples in 10 different runs using the *17-OH Progesterone ELISA*. The inter-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	0.66	2.20	4.91
SD (ng/ml)	0.1	0.18	0.31
CV (%)	14.9	8.0	6.4
n =	10	10	10

9.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to three different samples containing different amounts of endogenous analyte. All samples were then measured by the 17-OH-progesterone assay procedure. The percentage recoveries were determined by comparing expected and measured values of the samples.

Serum	Spiking	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Recovery %
1	- 2 ng/ml 4 ng/ml 6 ng/ml	0.64 2.55 3.69 4.95	- 2.64 4.64 6.64	- 97 % 80 % 75 %
2	- 2 ng/ml 4 ng/ml 6 ng/ml	0.66 2.57 4.16 5.47	- 2.66 4.66 6.66	- 97 % 89 % 82 %
3	- 2 ng/ml 4 ng/ml 6 ng/ml	2.66 5.68 7.39 9.87	- 4.66 6.66 8.66	- 122 % 111 % 114 %

9.6 Linearity

Three serum samples containing different amounts of analyte were assayed undiluted and diluted with the Standard matrix (Standard A). The percentage recovery was calculated by comparing the expected and measured values for 17-OH-progesterone.

Serum	Dilution	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Recovery %
1	- 1 in 2 1 in 4 1 in 8	21.06 10.00 4.98 2.49	- 10.53 5.26 2.63	- 95 % 95 % 95 %
2	- 1 in 2 1 in 4 1 in 8	15.06 7.84 4.54 2.33	- 7.53 3.77 1.88	- 104 % 120 % 124 %
3	native 1 in 2 1 in 4 1 in 8	23.38 11.43 5.49 2.49	- 11.69 5.84 2.92	- 98 % 94 % 85 %

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 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of 17-OH-progesterone in a sample.

11. REFERENCES

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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!