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Instructions for use

Progesterone rat/mouse ELISA

REF **AR E-8700**

Σ 96

+2 +8 °C

RUO For Research use only-
Not for use in diagnostic procedures

PROGESTERONE RAT/MOUSE ELISA

INTRODUCTION

INTENDED USE

The **Progesterone rat/mouse ELISA** is a competitive immunoassay for the quantitative measurement of progesterone in rat and mouse serum or plasma. For research use only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

Progesterone (4-pregnene-3, 20-dione) is a C21 steroid hormone containing a keto-group (at C-3) and a double bond between C-4 and C-5. Like other steroids, it is synthesized from cholesterol via a series of enzyme-mediated steps (1). Progesterone is a female sex hormone of primary importance in ovulation, fertility and menopause. It is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy (2). The rate of progesterone secretion may be affected by the degree of progestational activity of the uterus and the level of circulating LH (3). Analyses suggest that progesterone acts as an anti-glucocorticoid in rat adipose tissue in vivo, attenuating the glucocorticoid effect on adipose tissue metabolism (4). Furthermore it could be demonstrated that progesterone alone may be a valuable agent for management of postmenopausal osteoporosis (5).
In female rodents, the determination of progesterone is a useful marker in evaluating and monitoring the state of the reproductive functions and pregnancy as well.

PRINCIPLE

The **Progesterone rat/mouse ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. An unknown amount of progesterone present in the sample and a defined amount of progesterone conjugated to horseradish peroxidase compete for the binding sites of progesterone antiserum coated to the wells of a microplate. After incubation on a shaker the microplate is washed four times. After addition of the substrate solution the concentration of progesterone is inversely proportional to the optical density measured.

WARNINGS AND PRECAUTIONS

- For professional use only.
- 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.
- Do not mix reagents of different lots. Do not use expired reagents.
- The microplate contains snap-off strips. Unused wells must be stored at 2 – 8°C in the sealed foil pouch and used in the frame provided.
- Avoid contact with Stop Solution. It may cause skin irritation and burns.
- Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
- Change pipette tips between samples and reagents to avoid carry over contamination.
- 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.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Assay reagents contain Thimerosal against microbial growth. In case of contact with eyes or skin, flush immediately with water.
- All reagents should be at room temperature (21-26°C) before use. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
- TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

REAGENTS

REAGENTS PROVIDED

AR E-8731 96 **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells; ready to use;
Wells coated with polyclonal anti-progesterone antibody.

Calibrators - ready to use.

Cat. no.	Symbol	Calibrator	Concentration	Volume/Vial
AR E-8701	STANDARD A	**Calibrator 0**	0 ng/ml	0.5 ml
AR E-8702	STANDARD B	**Calibrator 1**	0.4 ng/ml	0.5 ml
AR E-8703	STANDARD C	**Calibrator 2**	1.5 ng/ml	0.5 ml
AR E-8704	STANDARD D	**Calibrator 3**	6.5 ng/ml	0.5 ml
AR E-8705	STANDARD E	**Calibrator 4**	25 ng/ml	0.5 ml
AR E-8706	STANDARD F	**Calibrator 5**	100 ng/ml	0.5 ml

AR E-8713 INC-BUFF **Incubation Buffer,** 1 vial 7 ml, ready to use;

AR E-8740 CONJUGATE **Enzyme Conjugate**, 1 vial, 11 ml, ready to use;
Progesterone conjugated to horseradish peroxidase.

AR E-0055 SUBSTRATE **Substrate Solution**, 1 vial, 22 ml, ready to use;
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

AR E-0080 STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;
contains 2 N Hydrochloric Acid solution.

AR E-0030 WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10X concentrated);
see „Preparation of Reagents".

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge
- A microtiter plate reader capable for endpoint measurement at 450 nm
- Microplate mixer operating more than 600 rpm
- 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

REAGENT PREPARATION

All reagents should be at room temperature before use.

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

STORAGE CONDITIONS

When stored at 2°C to 8°C all reagents are stable until expiration date. Do not use reagents beyond this date.
The Stop Solution is stable up to 2 months after opening.
The Wash Solution is stable for 3 months after dilution.
Protect divisible Microplate from moisture. Store together with desiccant and carefully sealed in the foil bag.
Opened reagents must be stored at 2°C to 8°C.

SPECIMEN

For determination of Progesterone rat/mouse **serum** and **plasma** can be used. The procedure calls for 25 µl matrix per well. The samples should assay immediately or aliquot and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain rat/mouse Progesterone concentrations higher than the highest calibrator (100 ng/ml) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results.

Please note: The use of plasma as specimen can result in a diminished precision of this assay.

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 and 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.

ASSAY PROCEDURE

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **25 µl** of each **Calibrator** and **Sample** with new disposable tips into appropriate wells.
3. Dispense **50 µl** of **Incubation Buffer** into each well.
4. Add **100 µl Enzyme Conjugate** into each well.
5. Incubate for **1 hour** at room temperature on a microplate mixer.

   Important Note:
   Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7. Add **200 µl** of **Substrate Solution** to each well.
8. Incubate without shaking for **30 minutes** in the dark.
9. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
10. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of calibrators, controls and patient 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 calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.
5. The concentration of the samples can be determined directly from this calibrator 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.

Conversion to SI units:
Progesterone (ng/ml) x 3.18 = nmol/l

Example of Typical Calibrator Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Absorbance Units
Calibrator 0 (0 ng/ml)	2.438
Calibrator 1 (0.4 ng/ml)	1.981
Calibrator 2 (1.5 ng/ml)	1.618
Calibrator 3 (6.5 ng/ml)	1.180
Calibrator 4 (25 ng/ml)	0.731
Calibrator 5 (100 ng/ml)	0.320

PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY

The lowest analytical detectable level of progesterone that can be distinguished from the Zero Calibrator is 0.04 ng/ml at the 2SD confidence limit.

SPECIFICITY

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to progesterone.

Steroid	% Cross reaction
Androstenedione	< 0.1
Androsterone	< 0.1
Corticosterone	0.3
11-Desoxycorticosterone	1.8
5α-Dihydrotestosterone	< 0.1
Estradiol	< 0.1
Estriol	< 0.1
17α-Hydroxyprogesterone	0.6
Prednisolone	< 0.1
Prednisone	< 0.1
Pregnenolone	5.5
Testosterone	0.14

REPRODUCIBILITY

Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 5 serum samples within one run. The within-assay variability is shown below:

Mean (ng/ml)	12.1	15.0	17.9	61.9	80.0
SD	1.2	1.4	1.4	4.0	3.7
CV (%)	9.9	9.7	8.0	6.4	4.6
n =	20	20	20	20	20

Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of 3 serum samples in 10 different assay runs.

Mean (ng/ml)	11.5	36.2	80.5
SD	0.9	2.2	4.8
CV (%)	7.6	6.0	6.0
n =	10	10	10

RECOVERY

Using the Calibrator matrix a spiking solution was prepared (1000 ng/mL). Aliquots of 5, 10 and 15 µL, respectively, were spiked into 495 µL, 490 µL and 485 µL of three different sera, leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by the rat Progesterone assay procedure.

Serum	**Spiking Solution**	**Observed (0)**	**Expected (E)**	**O/E %**
1	-	7,6	-	-
	A	16,1	17,6	91%
	B	25,2	27,6	91%
	C	34,1	37,6	91%
2	-	11,2	-	-
	A	17,4	21,2	82%
	B	31,3	31,2	100%
	C	34,0	41,2	83%
3	-	12,2	-	-
	A	22,8	22,2	103%
	B	31,9	32,2	99%
	C	36,0	42,2	85%

LINEARITY

Three native serum samples were assayed undiluted and diluted with the calibrator matrix.

Serum	**Dilution**	**Observed (0)**	**Expected (E)**	**O/E %**
1	native	70,7	-	-
	1 in 2	36,6	35,4	103%
	1 in 4	16,6	17,7	94%
	1 in 8	10,3	8,8	117%
2	native	72,7	-	-
	1 in 2	35,6	36,4	98%
	1 in 4	18,2	18,2	100%
	1 in 8	9,7	9,1	107%
3	native	55,7	-	-
	1 in 2	28,0	27,9	100%
	1 in 4	14,7	13,9	105%
	1 in 8	7,3	7,0	104%

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.

DRUG INTERFERENCES

Until now no substances (drugs) are known influencing the measurement of rat or mouse progesterone in serum. Lipemic and haemolysed samples can cause false results.

REFERENCES

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4. Pepe G.J. and Rothchild I (1974): A Comparative Study of Serum Progesterone Levels in Pregnancy and in Various Types of Pseudopregnancy in the Rat; *Endocrinology* July 1, 1974 vol. 95 no. 1 275-279
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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!