Mouse/Rat Progesterone ELISA

For the quantitative determination of progesterone in rat or mouse plasma and serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 55-PROMS-E01
Size: 96 tests
Version: 5a-12/19 – ALPCO 2.1

1. Introduction

1.1. Intended Use

The Mouse/Rat Progesterone 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.

1.2. 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). Research suggests 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 researching and monitoring the state of the reproductive functions and pregnancy.

2. Principle of the Assay

The Mouse/Rat Progesterone 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.

3. Warnings and Precautions

1. This kit is strictly intended for research use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing of infectious agents.
3. 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.
4. 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.
5. Pipetting of samples and reagents must be performed 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 (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the subject samples will not be affected.
10. Never pipette by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable gloves when handling samples and reagents. Microbial contamination of reagents or samples 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 give slightly different results.
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 more information please refer to the Safety Data Sheets. The Safety Data Sheets for this product are available upon request directly from ALPCO.

4. Reagents

4.1. Reagents Provided

1. **Microtiter plate**, 12 x 8 (break apart) strips with 96 wells; Wells coated with polyclonal anti-progesterone antibody.

2. **Calibrator 0**, 1 vial, 0.3 mL, ready to use.

3. **Calibrator (Calibrator 1-5),** 5 vials, 0.3 mL each, ready to use; Concentrations: 0.4, 1.5, 6.5, 25, 100 ng/mL.

4. **Incubation Buffer,** 1 vial 7 mL, ready to use.

5. **Enzyme Conjugate**, 1 vial, 7 mL, ready to use; Progesterone conjugated to horseradish peroxidase.

6. **Substrate Solution**, 1 vial, 22 mL, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

7. **Stop Solution,** 1 vial, 7 mL, ready to use; contains 2 N Hydrochloric Acid solution.

8. **Wash Solution**, 1 vial, 50 mL (10X concentrated); see “Preparation of Reagents.”

4.2. Materials Required but Not Provided

- Centrifuge
- A microtiter plate reader capable of 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

4.3. 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 1X working Wash Solution is stable for at least 3 months at room temperature (18-25°C).

4.4. 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. After the first opening, the reagents are stable for 30 days if used and stored properly.
Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.
Protect TMB Substrate Solution from light.

4.5. Disposal of the Kit

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 Kit

In case of any severe damage of the test kit or components, ALPCO must be informed in writing, within one week of receiving the kit. Severely damaged single components should not be used for a test run and must be stored until a final solution has been found. After, they should be disposed according to the official regulations.

5. Sample

For determination of progesterone, rat/mouse **serum** and **plasma** can be used. The procedure calls for 10 µL of matrix per well. The samples should be assayed immediately or aliquoted 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 or a validated alternate buffer 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 a sample type can result in a diminished precision of this assay.

6. Assay Procedure

6.1. General Remarks

- All reagents and samples 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 disposable plastic pipette tips for each standard and sample 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.
- For internal quality control, it is suggested to use the Rat Control Set 55-DEV99RC. Please contact ALPCO for more information.

6.2. Assay Procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators, controls and samples in duplicate.
2. Dispense **10 µL** of each **Calibrator, Control, and Sample** with new disposable tips into appropriate wells.
3. Dispense **50 µL** of **Incubation Buffer** into each well.
4. Add **50 µL Enzyme Conjugate** into each well.
5. Incubate for **1 hour** at room temperature (18-25°C) on a microplate mixer (>600 rpm).
   Important Note: Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6. Discard the contents 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 tapping 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.

6.3. Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each calibrator against its concentration with absorbance values on the vertical (Y) axis and the 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-Parameter Logistics (4-PL) curve fit. 4-PL 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 must 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

6.3.1. 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)	2.508
Calibrator 1 (0.4 ng/mL)	2.253
Calibrator 2 (1.5 ng/mL)	1.932
Calibrator 3 (6.5 ng/mL)	1.311
Calibrator 4 (25 ng/mL)	0.678
Calibrator 5 (100 ng/mL)	0.312

7. Performance Characteristics

7.1. Analytical Sensitivity

The lowest analytical detectable level of progesterone that can be distinguished from the Zero Calibrator is 0.156 ng/mL at the 2SD confidence limit.

7.2. Analytical Specificity

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

Steroid	% Cross-reactivity
17-β-Estradiol	< 0.1%
Cortisol	< 0.1%
Estrone	< 0.1%
Pregnenolone	6.0%
Prednisone	< 0.1%
Prednisolone	< 0.1%
11-Deoxycortisol	0.8%
DHEA	0.4%
Testosterone	4.3%
Cortisone	< 0.1%
Estriol	< 0.1%
Corticosterone	< 0.1%
Dexamethasone	< 0.1%
11-Deoxycorticosterone	6.1%
Danazole	0.2%
17-Hydroxyprogesterone	3.0%
Androstenedione	2.5%

7.3. Reproducibility

7.3.1. Intra-Assay

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

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	3.23	11.43	40.31
SD	0.37	0.84	2.02
CV (%)	11.5	7.4	5.0
n =	20	20	20

7.3.2. Inter-Assay

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

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.98	9.60	33.82
SD	0.41	0.87	2.08
CV (%)	13.6	9.1	6.1
n =	10	10	10

7.4. 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 progesterone assay procedure.

Serum	Spiking Solution	Observed (O) (ng/mL)	Expected (E) (ng/mL)	O/E %
1	-	15.42	-	-
	A	21.76	25.42	86
	B	29.08	35.42	82
	C	35.57	45.42	78
2	-	20.16	-	-
	A	30.85	30.16	102
	B	41.70	40.16	104
	C	52.56	50.16	105
3	-	3.73	-	-
	A	13.41	13.73	98
	B	25.74	23.73	108
	C	35.60	33.73	106

7.5. Linearity

Three serum samples containing different amounts of progesterone were assayed undiluted and diluted with the calibrator matrix.

Serum	Dilution	Observed (O) (ng/mL)	Expected (E) (ng/mL)	O/E %
1	neat	19.52	-	-
	1 in 2	8.96	9.76	92%
	1 in 4	4.61	4.88	94%
	1 in 8	2.65	2.44	109%
2	neat	21.88	-	-
	1 in 2	11.28	10.94	103%
	1 in 4	5.93	5.47	108%
	1 in 8	3.06	2.74	112%
3	neat	23.27	-	-
	1 in 2	13.53	11.64	116%
	1 in 4	6.34	5.82	109%
	1 in 8	3.00	2.91	103%

8. Limitations of Procedure

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

8.1. Drug Interferences

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

9. Legal Aspects

9.1. Reliability Of Results

The assay must be performed exactly as the instruction for use indicates. Moreover, the user must strictly adhere to the rules of Good Laboratory Practices (GLP) 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 assay.

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 ALPCO.

9.2. Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one kit to another could negatively affect the intended results and validity of the overall assay run. Such modifications and/or exchanges invalidate any claim for replacement.

Regardless, in the event of any claim, ALPCO's responsibility is not to exceed the value of the kit. Any damage caused to the kit during transport is not subject to the liability of ALPCO.

10. References

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