Mouse/Rat Progesterone ELISA Kit

Enzyme Immunoassay for the quantification of Mouse/Rat Progesterone in serum and plasma.

Catalog number: ARG80657

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

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. 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. The rate of progesterone secretion may be affected by the degree of progestational activity of the uterus and the level of circulating LH. Analyses suggest that progesterone acts as an anti-glucocorticoid in rat adipose tissue in vivo, attenuating the glucocorticoid effect on adipose tissue metabolism. Furthermore it could be demonstrated that progesterone alone may be a valuable agent for management of postmenopausal osteoporosis. 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 OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Progesterone has been pre-coated onto a microtiter plate. Endogenous Progesterone of a sample competes with a Progesterone-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 Progesterone in the sample. After addition of the substrate solution, the

intensity of color developed is inversely proportional to the concentration of Progesterone in the patient sample. Progesterone concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 x 8 strips	4°C
Standard 0	0.5 ml (ready to use)	4°C
Standard 1-5 (0.4, 1.5, 6.5, 25, 100 ng/ml)	5 x 0.5 ml (ready to use)	4°C
Incubation Buffer	7 ml (ready to use)	4°C
HRP conjugated Progesterone	11 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml	4°C (Protect from light)
STOP solution	7 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP- Progesterone concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- **Samples**: 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.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **standards and samples** in duplicate into sample wells.
3. Add **50 µl** of **Incubation Buffer** into each well.
4. Add **100 µl** of **HRP conjugated Progesterone** into each well, mix well. Incubate for 1 hour at room temperature on a microplate mixer or shaker.
5. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1× Wash Buffer** (**350 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **200 µl** of **TMB substrate** to each well. Incubate for 30 minutes at room

temperature in dark.

7. Add 50 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately. It is recommended to read the wells within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear 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 IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

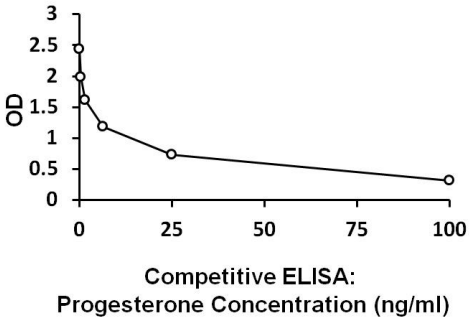
5. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6. Conversion to SI units:

Progesterone (ng/ml) x 3.18 = nmol/l

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.



QUALITY ASSURANCE

Specificity

Steroid	Crossreactivity (%)
Androstenedione	< 0.1
Aldosterone	< 0.1
Corticosterone	0.3
11-Deoxycorticosterone	1.8
5α-Dehydroepiandrosterone	< 0.1
Estriol	< 0.1
Estradiol	< 0.1
17α-Hydroxyprogesterone	0.6
Prednisolone	< 0.1
Prednisone	< 0.1
Pregnenolone	5.5
Testosterone	0.14

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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.72% and inter-assay precision was 6.53%.

Recovery

82-103%

Linearity

94-117%