Human Progesterone (free) ELISA Kit

Enzyme Immunoassay for the quantification of Progesterone (free) in Human saliva.

Catalog number: ARG82804

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Progesterone (P4) is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens, and is the major progestogen in the body. Progesterone has a variety of important functions in the body. It is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid.

In addition to its role as a natural hormone, progesterone is also used as a medication, such as in combination with estrogen for contraception, to reduce the risk of uterine or cervical cancer, and in menopausal hormone therapy and hormone replacement therapy (HRT) in cases where a woman's ovaries have been removed due to cancer or other physical issue. It was first prescribed in 1934. The Progesterone level in saliva represents the concentration of the active free Progesterone. [Provided by Wikipedia: Progesterone]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for Progesterone has been pre-coated onto a microplate. Progesterone containing samples, Controls or Standards and a Progesterone -HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free progesterone compete for the antibody binding sites. After incubation, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of Progesterone present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Progesterone in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C in the dark. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to F (0, 10, 50, 150, 600 / 2400 pg/mL)	1 mL each (ready to use)	4°C
Control 1 & 2	1 mL each (ready to use)	4°C
Progesterone-HRP Conjugate	26 mL	4°C
40X Wash Buffer	30 mL	4°C
TMB substrate	25 mL (ready to use)	4°C (protect from light)
STOP solution	14 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Saliva collection device
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (21-26°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 40X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.

- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Saliva:

1. Collect saliva sample with a centrifuge glass tube (contain a plastic straw).
2. Store at-20°C for at least overnight.
3. Centrifuge again at 10000 g for 5-10 minutes.
4. The clear supernatant must be transferred into a fresh tube.
5. Use supernatant for the assay.

Note:

1. Samples containing sodium azide should not be used in the assay.
2. Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling.
3. It is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.
4. If there is visible blood contamination of the patient specimen, it should be discarded, rinse the sampling device with distilled water, wait for 10 minutes and take a new sample.
5. Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimens stored for a longer time should be frozen only once at-20°C prior to assay. The supernatant should be frozen only once.

Thawed supernatant should be inverted several times prior to testing!

6. For samples with Progesterone concentration greater than 2400 pg/ml, the sample can be diluted with Standard A and re-assayed.
7. For the calculation of the concentrations this dilution factor has to be taken into account.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 40X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 30 mL of 40X Wash Buffer into 1170 mL of distilled water to a final volume of 1200 mL) The 1X Wash Buffer is stable for up to 2 weeks at room temperature.

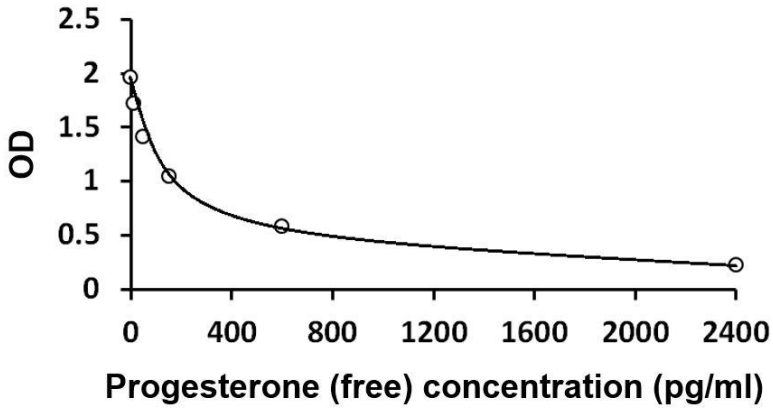
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **Standards, Controls and prepared samples** into the appropriate wells of the Antibody Coated Microplate.
2. Add **200 µL** of **Progesterone-HRP Conjugate** into all wells. Thoroughly mix for **10 seconds**. It is important to have a complete mixing in this step.
3. Incubate at **RT** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer** (400 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.
5. Add **200 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **15 minutes** at **RT** on a microplate shaker.
6. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Progesterone (free) ELISA kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or 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. 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.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. For conversion: pg/mL x 3.18 = pmol/L
6. Reference value:

Group / Age		Salivary progesterone (pg / mL)
Women	21-50 years, Follicular phase (n=40)	19.6-86.5
	21-50 years, Luteal phase (n=40)	99.1-332.6
	51-75 years Postmenopausal (n=40)	6.0-56.4
Men	(n=50)	1.1-44.4

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human Progesterone (free) ELISA kit is 1.1 pg/mL.

Specificity

Substance	Cross Reactivity (%)
Progesterone	100
Desoxycorticosterone	1.1
Pregnenolone	0.35
17α-Hydroxyprogesterone	0.9
Corticosterone	0.2
11-Desoxycortisol	0.1
Estriol	0.0
Estradiol 17β	0.0
Testosterone	0.2
Cortisone	< 0.1
DHEA-S	0.0
Cortisol	2.6
Androstendione	0.4
DHEA	0.0
Estron	0.0

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was ≤ 8.1% and CV value of inter-assay precision was ≤ 10.7%.

Recovery

89.1-113.1%