



## **Human Progesterone ELISA Kit**

Enzyme Immunoassay for the quantification of Progesterone in Human serum and plasma (EDTA, lithium heparin, citrate).

Catalog number: ARG82795

Package: 96 wells

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For research use only. Not for use in diagnostic procedures.

## **TABLE OF CONTENTS**

<b>SECTION</b>	<b>Page</b>
INTRODUCTION.....	3
PRINCIPLE OF THE ASSAY.....	4
MATERIALS PROVIDED & STORAGE INFORMATION.....	5
MATERIALS REQUIRED BUT NOT PROVIDED.....	5
TECHNICAL NOTES AND PRECAUTIONS.....	6
SAMPLE COLLECTION & STORAGE INFORMATION.....	7
REAGENT PREPARATION.....	8
ASSAY PROCEDURE.....	8
EXAMPLE OF TYPICAL STANDARD VALUES.....	9
CALCULATION OF RESULTS.....	10
QUALITY ASSURANCE.....	11

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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. [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 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 at room temperature, 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.

## Human Progesterone ELISA kit ARG82795

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### MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to G (0, 0.3, 1.25, 2.5, 5, 15, 40 ng/mL)	1 mL each (ready to use)	4°C
Progesterone-HRP Conjugate	25 mL (ready to use)	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
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- 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 (20-26°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.

## Human Progesterone ELISA kit ARG82795

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- 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.

**Serum:** Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

**Plasma:** Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

#### **Note:**

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 7 days at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.
5. If in an initial assay, a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with Standard 0.

### REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 40X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 15 mL of 40X Wash Buffer into 585 mL of distilled water to a final volume of 600 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, 20-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **25 µL** of **Standards, Controls and samples** into the appropriate wells of the Antibody Coated Microplate.
2. Incubate at **RT** for **5 minutes** on a microplate shaker.
3. 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.**
4. Incubate at **RT** for **60 minutes** on a microplate shaker.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 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, including the blank wells. Incubate in the dark for **15 minutes** at **RT** on a microplate shaker.
7. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank



## Human Progesterone ELISA kit ARG82795

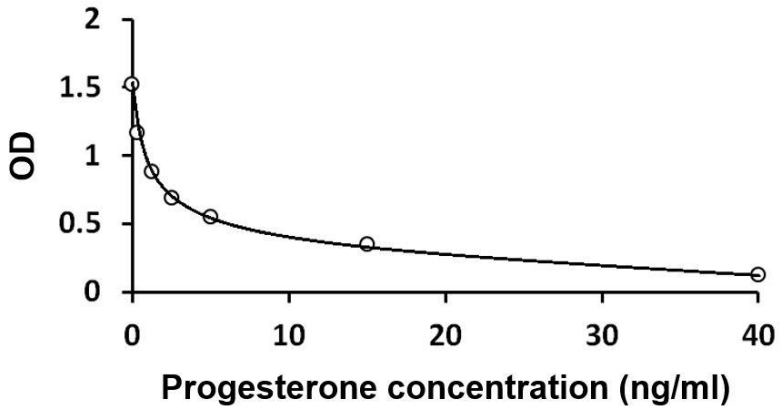
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wells. The color of the solution should change from blue to yellow.

8. 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 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. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. Conversion: Progesterone 1 ng/ml = 3.18 nmol/L

## Human Progesterone ELISA kit ARG82795

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### QUALITY ASSURANCE

#### Sensitivity

0.045 ng/mL.

#### Specificity

Substance	Cross Reactivity (%)
Progesterone	100
17 $\alpha$ OH Progesterone	0.3
Estriol	< 0.1
Estradiol 17 $\beta$	< 0.1
Testosterone	< 0.1
11-Desoxycorticosterone	1.1
DHEA-S	< 0.1
Corticol	< 0.1
Corticosterone	0.2
Pregnenolone	0.35
Cortison	< 0.1
11-Desoxycortisol	0.1

#### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.4-7% and CV value of inter-assay precision was 4.3-10%.

#### Recovery

90.9-112%