Package Insert

# Progesterone ELISA Assay Kit

Catalog Number:

PRG31-K01 (1 x 96 wells)

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 8.0 (10 MAY 24)*

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## INTENDED USE

The Eagle Biosciences Progesterone ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of progesterone in human serum. The Eagle Biosciences Progesterone ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## INTRODUCTION

Progesterone is a C-21 female sex steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced in low levels. It increases to the LH peak and then sharply rises 3 to 4 days later to higher levels, remaining elevated through the 10th to 12th days after the LH peak. Then there is a sharp decline to the low levels of the follicular phase. It is responsible for the induction of the cyclic changes in the endometrium of the uterus allowing implantation and successful growth of the fertilized ovum and maintenance of pregnancy. Progesterone measurements are used in documenting ovulation and in the management of difficulties during the first trimester of pregnancy. Levels of progesterone may be useful in the evaluation of sterility due to luteal phase defects, prediction of impending abortion, and the diagnosis of ectopic pregnancy. Normal values of progesterone may be affected by drugs such as, oral contraceptives, super ovulatory drugs, estrogen replacement therapy medication, and GnRH analogues. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence serum progesterone values.

## PRINCIPLE OF THE ASSAY

The Progesterone ELISA is a competitive immunoassay. Competition occurs between progesterone present in calibrators, controls, specimen samples and an enzyme-labeled antigen (HRP conjugate) for a limited number of anti-progesterone antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of testosterone present. Following an incubation, the enzymatic reaction color to a yellow color. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of testosterone in specimen samples and controls can be directly read.

## PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a Do not pipette by mouth.
    b Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c Wear protective clothing and disposable gloves.
    d Wash hands thoroughly after performing the test.
    e Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

4. Do not use this kit beyond the expiry date stated on the label. 5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >60 pg/mL. If further dilution and retesting is required, only Calibrator A may be used to dilute saliva samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.

25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.

## SAFETY CAUTIONS AND WARNINGS

### BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain processed human serum/plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV ½ and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

### CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

## SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

### Specimen Collection & Storage

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of venous blood into an appropriately labeled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

## Specimen Pre-Treatment

Specimen pre-treatment is not required.

## REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 25 µL.
2. Calibrated multi-channel pipette to dispense 50 µL, 100 µL, and 150 µL.
3. Calibrated multi-channel pipettes to dispense 300 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
    a. Orbital shaker (3 mm diameter) set to 600 rpm or
    b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.

## REAGENTS PROVIDED

**1. Microplate**

| | |
|---|---|
| Contents: | One anti-progesterone polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant. |
| Format: | Ready to Use |
| Storage: | 2-8°C |
| Stability: | Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. |

**2. HRP Conjugate**

| | |
|---|---|
| Contents: | One bottle containing Progesterone-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative |
| Format: | Concentrated; Requires Preparation |
| Storage: | 2-8°C |
| Stability: | Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. |
| Preparation of HRP Conjugate Working Solution: | **Dilute 1:101** in assay buffer before use (e.g., 20µL of conjugate concentration in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over. |

**3. Calibrator A - F**

Contents: Six bottles of calibrator containing specified progesterone concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with define quantities of progesterone.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations.
Concentrations: 0, 0.3, 1, 5, 20, 60 ng/mL

Format: Ready to Use

Volume: Calibrator A: 2.0 mL/bottle
Calibrator B-F: 0.5 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

**4. Control 1 -2**

Contents: Two bottles of control containing different progesterone concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of progesterone. Refer to the QC certificate for the target values and acceptable ranges.

Format: Ready to Use

Volume: 0.5 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

**5. Assay Buffer**

Contents: One bottle containing a protein-based buffer with a non-mercury preservative

Format: Ready to Use

Volume: 15 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

**6. TMB Substrate**

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

| | |
|---|---|
| Format: | Ready to Use |
| Volume: | 16 mL/bottle |
| Storage: | 2-8°C |
| Stability: | Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. |

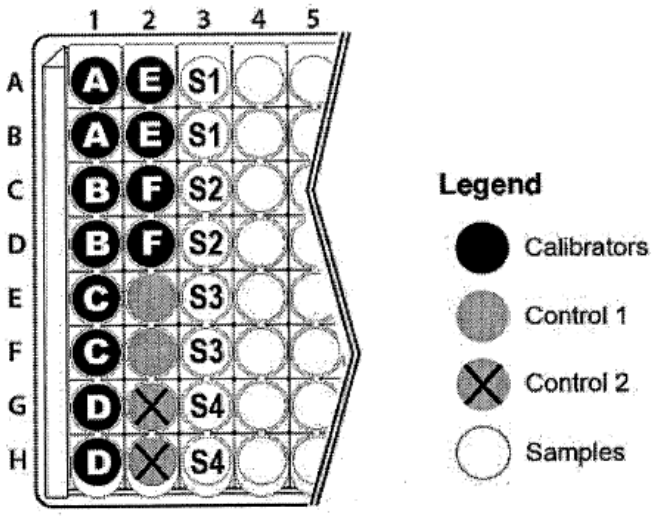
**7. Stopping Solution**

| | |
|---|---|
| Contents: | One bottle containing 1M sulfuric acid. |
| Format: | Ready to Use |
| Volume: | 6 mL/bottle |
| Storage: | 2-8°C |
| Stability: | Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. |
| Safety: | Refer to product SDS. |

**8. Wash Buffer Concentrate**

| | |
|---|---|
| Contents: | One bottle containing buffer with a non-ionic detergent and a non-mercury preservative. |
| Format: | Concentrated; Requires Preparation |
| Volume: | 50 mL/bottle |
| Storage: | 2-8°C |
| Stability: | Unopened: Stable until expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use. |
| Preparation of Wash Buffer Working Solution: | Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water. |

## RECOMMENDED ASSAY LAYOUT




## ASSAY PROCEDURE

Specimen Pretreatment: None

All kit components, controls, and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (See section *Reagents Provided, HRP Conjugate Concentration, Wash Buffer Concentrate*).
3. **Plan** the microplate wells to be used for calibrators, controls, and samples. See *Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 25 µL** of each calibrator, control, and pre-treated specimen sample into assigned wells.
5. **Pipette 100 µL** of the HRP conjugate into each well (the use of a multi-channel pipette is recommended).
6. **Incubate** the microplate on a microplate shaker** for **60 minutes** at room temperature.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as state below.
    a **Automatic:** Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL /well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

b **Manually:** For manual washing, perform a **3-cycle** wash using **300 µL /well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
9. **Incubate** the microplate on a microplate shaker** for **10-20 minutes** at room temperature.
10. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for the addition of TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

**See Section *Reagents and Equipment Needed But Not Provided* for microplate shaker options

## CALCULATIONS

1. Calculate the mean optical density of each calibrator, control, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 60 ng/mL and needs to be diluted and retested, then dilute it with calibrator A not more than 1:8. The result obtained should be multiplied by the dilution factor.

## QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator / OD of calibrator A) X 100.
2. The values obtained for the kit controls are within the acceptable ranges as stated in the QC Certificate.
3. The results of any external controls that were used meet the acceptable ranges.

## TYPICAL TABULATED DATA

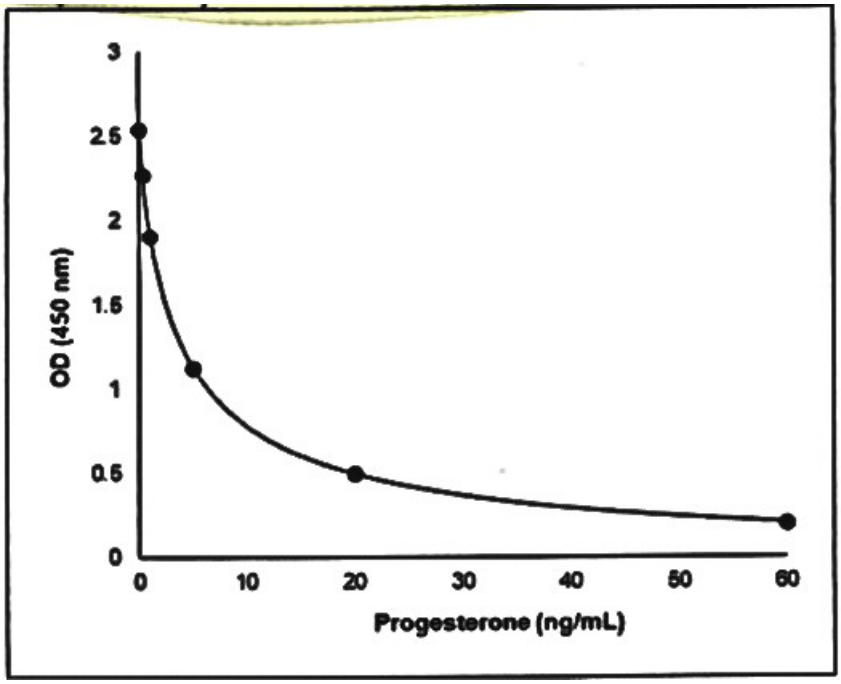
Sample data only. Do not use to calculate results.

| Calibrator | Mean OD | % Binding | Value (ng/mL) |
|---|---|---|---|
| A | 2.538 | 100 | 0 |
| B | 2.274 | 90 | 0.3 |

| | | | |
|---|---|---|---|
| C | 1.909 | 75 | 1 |
| D | 1.124 | 44 | 5 |
| E | 0.502 | 20 | 20 |
| F | 0.200 | 8 | 60 |
| Unknown | 0.853 | - | 8.6 |

## TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.




## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Progesterone ELISA kit is 0.1 ng/mL.

### SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Progesterone ELISA kit with Progesterone cross-reacting at 100%.

| Steroid | % Cross Reactivity |
|---|---|
| Progesterone | 100 |
| 11a-OH-Progesterone | 100 |
| Deoxycorticosterone | 1.7 |
| 17-OH-Progesterone | 0.4 |
| 5a-Androstane-3b, 17b-diol | 0.3 |
| Corticosterone | 0.3 |
| Pregnenolone | 0.2 |

The following steroids were tested but cross-reacted at less than 0.1%: Cortisol, Cortisone, Danazol, DHEAS, Estradiol, 5β-Pregnan-3α, 17α, 21α-triol-20-one, 5β-Pregnan-3α, 17-diol-20one, Pregnan-3α, 20α-diol and Testosterone.

## PRECISION

### Intra-Assay Precision

Two serum samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

| Sample | Mean | SD | CV % |
|---|---|---|---|
| 1 | 1.89 | 0.20 | 10.6 |
| 2 | 14.24 | 1.45 | 10.2 |

### Inter-Assay Precision

Two samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

| Sample | Mean | SD | CV % |
|---|---|---|---|
| 1 | 2.63 | 0.33 | 12.6 |
| 2 | 10.15 | 1.04 | 10.2 |

## LINEARITY

Two serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below.

| Sample | Observed Result | Expected Result | Recovery % |
|---|---|---|---|
| 1 | 10 | - | - |
| 1:2 | 5.3 | 5 | 106 |
| 1:4 | 2.16 | 2.5 | 86 |
| 1:8 | 1.38 | 1.25 | 110 |
| 1:16 | 0.59 | 0.63 | 95 |
| 2 | 20 | - | - |
| 1:2 | 10 | 9.1 | 91 |
| 1:4 | 5 | 4.2 | 84 |
| 1:8 | 2.5 | 2.0 | 81 |
| 1:16 | 1.25 | 1.1 | 88 |

## RECOVERY

Spiked samples were prepared by adding defined amounts of progesterone to two serum samples. The results (in ng/mL) are tabulated below.

| Sample | Obs. Result | Exp. Result | Recovery % |
|---|---|---|---|
| 1 Unspiked | 20 | - | - |
| + 5.0 | 13 | 12.5 | 104 |
| + 20.0 | 16 | 20 | 80 |
| + 60.0 | 31 | 40 | 78 |
| 2 Unspiked | 45 | - | - |
| + 5.0 | 31 | 25 | 124 |
| + 20.0 | 35 | 32.5 | 108 |
| + 60.0 | 48 | 52.5 | 91 |

## COMPARATIVE STUDIES

The Progesterone ELISA kit (Kit A) was compared against a competitor's coated tube RIA kit (Kit B). The results (in ng/mL) are tabulated below:

| Group | Kit A Mean | Kit B Mean |
|---|---|---|
| 33 Samples | 3.05 | 2.76 |

## REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. ND = Not Detectable

| Group | Range (ng/mL) | Abs. Range (ng/mL) |
|---|---|---|
| Males | 0.53 | ND-1.35 |
| Females | 8.37 | ND-70.0 |
| Postmenopausal Female | 0.46 | ND-4.0 |

## REFERENCES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## Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.