EAGLE BIOSCIENCES

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 (23JAN23)

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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 Eagle Biosciences Progesterone ELISA Assay Kit is a competitive immunoassay. Competition occurs between progesterone present in standards, 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 progesterone present. Following and incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of progesterone 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).
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.

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4. Do not use the 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 standard curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum 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 procedural techniques or pipetting, incomplete washing, or 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 colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
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 should be reported as >60 ng/mL and must not be diluted. Dilution will alter the existing equilibrium and 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, standard, and control
20. To prevent the 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 shaker used can influence the optical densities and

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test results. If a different type of shaker 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. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. This test is not intended to be used for screening purposes
2. This test is not intended for home testing or self-testing
3. The kit is calibrated for the determination of progesterone in human serum. The kit is not calibrated for the determination of progesterone in other specimens of human or animal origin
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis or for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drug and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. If contacted with any of these reagents, wash with plenty of water and refer to the 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 labelled tube and allow it to clot. Centrifuge at room temperature and carefull 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 pipettes to dispense 50, 100, 150, and 300 µL (if washing manually)

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3. Disposable pipette tips
4. Automatic microplate washer (recommended).
5. Distilled or deionized water
6. 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.
7. Calibrated absorbance microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

1. Microplate

Contents: One 96-well (12x8) polyclonal antibody-coated 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 Concentrate (101x)

Contents: One bottle containing Progesterone-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format: Concentrated; Requires Preparation
Volume: 0.4 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label
After opening: Stable for four weeks
After Preparation: Discard unused diluted reagent
Preparation: **Dilute 1:101** in assay buffer before use (eg. 20 µL of HRP conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. Standard A - F

Contents: Six bottles containing specified progesterone concentrations in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of progesterone.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Standard	Concentration	Volume/Vial
Standard A	0 ng/mL	2.0 mL
Standard B	0.3 ng/mL	0.5 mL
Standard C	1 ng/mL	0.5 mL
Standard D	5 ng/mL	0.5 mL
Standard E	20 ng/mL	0.5 mL
Standard F	60 ng/mL	0.5 mL

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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

4. Controls 1 – 2

Contents: Two bottles of control containing different progesterone concentrations in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum 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. Wash Buffer Concentrate (10x)

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 the expiry date printed on the label
After opening: Stable for four weeks
After preparation: Stable for two weeks
Preparation: **Dilute 1:10** in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer

Contents: One bottle containing 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

7. 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

8. Stopping Solution – Ready to Use

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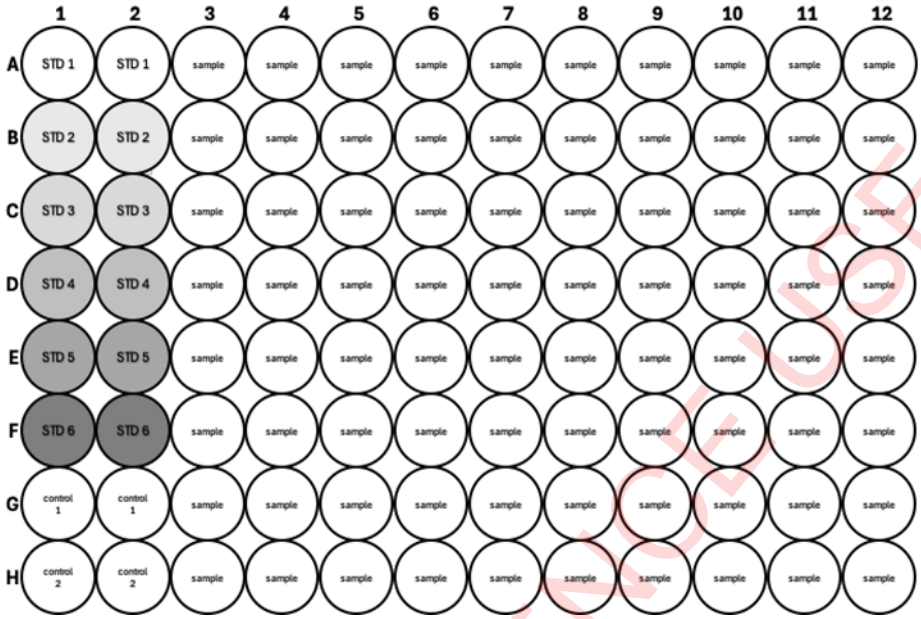
Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label After opening: Stable for four weeks

RECOMMENDED ASSAY LAYOUT



***** *Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, 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 working solutions of the progesterone-HRP conjugate and wash buffer.
3. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
4. **Pipette 25 µL** of each standard, control, and specimen sample into appropriate wells.
5. **Pipette 100 µL** of the HRP conjugate working solution 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 automated microplate washer (preferred) or manually as stated below.

   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.

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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 waste 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 addition of the 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.

QUALITY CONTROL

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

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

CALCULATIONS

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

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Standard	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 STANDARD CURVE

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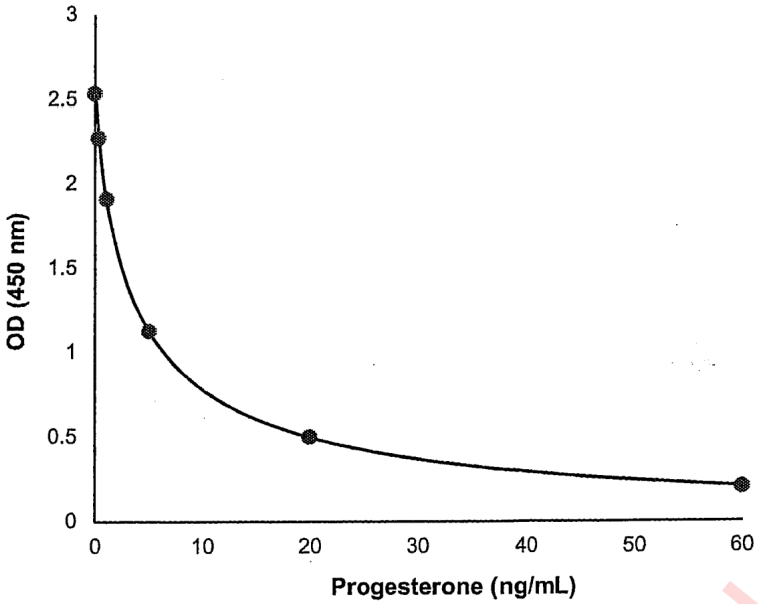

PERFORMANCE CHARACTERISTICS

Sensitivity

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

Specificity (Cross-Reactivity)

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

Steroid	% Cross Reactivity
Progesterone	100
11α-OH-Progesterone	100
Deoxycorticosterone	1.7
17-OH-Progesterone	0.4
5α-Androstane-3β, 17β-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-20-one, Pregnan-3α, 20α-diol and Testosterone.

Intra-Assay Precision

Two serum samples were assayed ten times each on the same standard 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

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Two serum 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

Recovery

Spiked samples were prepared by adding defined amounts of progesterone to two serum samples (1:1). 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

Linearity

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

Sample	Obs. Result	Exp. 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

COMPARATIVE STUDIES

The Direct Progesterone ELISA kit (Kit A) was compared with a competitors 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 assays each laboratory should collect data and establish their own range of expected normal values.

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

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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.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

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.

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