EAGLE BIOSCIENCES

Progesterone Saliva ELISA Assay Kit

Catalog Number:
PRG32-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 7.0 (02 APR 24)

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INTENDED USE

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

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

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. Next there is a sharp decline to the low levels of the follicular phase. Progesterone 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 useful 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. Drugs such as, oral contraceptives, superovulatory drugs, estrogen replacement therapy medication, and GnRH analogues may affect normal values of progesterone. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence salivary progesterone values. The determination of salivary progesterone combines a highly sensitive technique and non-invasive sample collection that is of value in clinical and research studies.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of progesterone in the sample. A set of standards is used to plot a standard curve from which the amount of progesterone in patient 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 >100 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.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of progesterone in human saliva. The kit is not calibrated for the determination of progesterone in serum, plasma or other specimens of human or animal origin.
2. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
3. Only calibrator A may be used to dilute any high saliva samples. The use of any other reagent may lead to false results.
4. This kit is for research use only and should not be used for diagnostic procedures.

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. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 1 mL of saliva is required per duplicate determination. Collect 4–5 mL of saliva into a clean glass tube* between 7–10 am without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4°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.

***Do not use cotton or polyester rolls or plastic collection tubes for collecting saliva samples in this assay, since it has been well established that false elevated results will occur.**

SPECIMEN PRETREATMENT

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed, heated at 60°C for 1 hour, and then centrifuged. The supernatants

are to be collected and poured into freshly labelled tubes. Do not use blood-contaminated specimens. If samples are to be used at a later date store frozen.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Deionized water
4. Plate shaker
5. Bench top centrifuge
6. Water bath set to 60°C
7. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Rabbit Anti-Progesterone Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   - Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **Progesterone-Horseradish Peroxidase (HRP) Conjugate Concentrate** — Requires Preparation X100
   - Contents: Progesterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 300 µL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 1200 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **Progesterone Saliva Calibrators** — Ready To Use
   - Contents: Six vials containing progesterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity progesterone.

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

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/mL	2.0 mL
Calibrator B	20 pg/mL	0.5 mL
Calibrator C	100 pg/mL	0.5 mL
Calibrator D	500 pg/mL	0.5 mL
Calibrator E	2000 pg/mL	0.5 mL
Calibrator F	5000 pg/mL	0.5 mL

   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** — Ready To Use
   - Contents: Two vials containing progesterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of progesterone. Refer to vial labels for the acceptable range.
   - Volume: 0.5 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** — Requires Preparation X10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   - Volume: 50 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - 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** – Ready to Use*
   - Contents: One bottle containing protein-based buffer with a non-mercury preservative.
   - Volume: 15 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

   *Warm to completely dissolve before use

7. **TMB Substrate** – Ready to Use
   - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   - Volume: 16 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

8. **Stopping Solution** – Ready to Use
   - Contents: One bottle containing 1M sulfuric acid.
   - Volume: 6 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: Freezing, Heating at 60 °C for 1 hour & Centrifugation

All reagents must reach room temperature before 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. Prepare working solutions of the progesterone-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette **50 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette **100 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. **Incubate** on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. **Wash** the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette **150 µL** of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 5000 pg/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

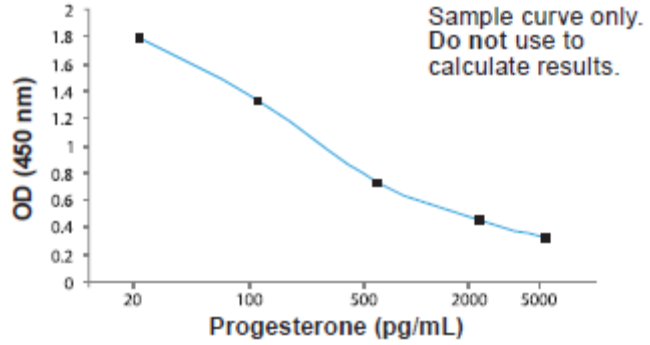
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (pg/mL)
A	2.252	2.320	2.286	0
B	1.795	1.768	1.782	20
C	1.352	1.322	1.337	100
D	0.730	0.736	0.733	500
E	0.453	0.451	0.452	2000

F	0.341	0.307	0.324	5000
Unknown	0.915	0.919	0.917	300

TYPICAL CALIBRATOR CURVE



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 Saliva ELISA kit is 20 pg/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Progesterone Saliva 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-20-one, Pregnan-3α, 20α-diol and Testosterone.

INTRA-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	32.93	4.39	13.3
2	78.73	4.63	5.9
3	302.67	22.30	7.37

INTER-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	30.83	3.90	12.7
2	75.03	7.73	7.7
3	241.06	26.23	10.9

RECOVERY

Spiked samples were prepared by adding defined amounts of progesterone to two patient saliva samples (1:1). The results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	4.38	-	-
+100	58.93	52.19	112.9
+500	240.57	252.19	95.4
+2000	851.70	1002.19	85.0
2 Unspiked	7.49	-	-
+100	46.27	53.75	86.1
+2000	894.58	1003.75	89.1
+5000	2694.49	2503.75	107.6

LINEARITY

Three patient saliva samples were diluted with calibrator A. The results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	1005.66	-	-
1:2	473.10	502.83	94.1
1:4	218.29	251.41	86.8
1:8	115.63	125.71	92.0
2	1462.5	-	-
1:2	700.48	731.25	95.8
1:4	327.69	365.62	89.6
1:8	172.12	182.81	94.1
3	2279.9	-	-
1:2	1061.0	1139.95	93.1
1:4	497.67	569.98	87.3
1:8	239.59	284.99	84.1

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (pg/mL)
Females	
Follicular Phase	<100
Luteal Phase	100-500
Postmenopausal	<500

REFERENCES
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2. Walker RF, et al. Characterization of Profiles of Salivary Progesterone During the Luteal Phase of Fertile and Subfertile Women. J Endocrinol. 1985; 104(3):441–6.
3. Zorn JR, et al. Salivary Progesterone as an Index of the Luteal Function. Fertil Steril. 1984; 41(2):248–53.
4. Vining RF, et al. Hormones in Saliva: Mode of Entry and Consequent Implications for Clinical Interpretation. Clin Chem. 1983; 29(10):1752–6.
5. Lenton EA, et al. Measurement of Progesterone in Saliva: Assessment of the Normal Fertile Range Using Spontaneous Conception Cycles. Clin Endocrinol (Oxf). 1988; 28(6):637–46.
6. Heasley RN, Thompson W. Salivary Progesterone Measurements in the Normal Menstrual Cycle. Ir J Med Sci. 1986; 155:(1)19–22.
7. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. Gynecol Obstet Invest. 1995; 40(2):139–40.

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.