Estradiol Saliva ELISA Kit

Catalog Number:
EST32-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
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EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Estradiol Saliva ELISA kit is designed for the quantitative measurement of 17β-Estradiol in human saliva samples. This assay is intended for research use only and should not be used for diagnostic procedures.

INTRODUCTION

17β-Estradiol (E2) is a steroid hormone produced mainly by the Graafian follicle of the ovary in females and in small amounts buy the testes in male subjects. E2 is biologically the most active and naturally produced human estrogens. The majority of E2 (98%) is bound to the sex hormone binding globulin (SHBH) and to the lesser extent to the other serum proteins such as albumin. Only a small fraction circulates in the free form is conjugated to sulfates and glucuronides. In non-pregnant womenthere is a cyclic variation in the concentration of E2, the highest values being measured usually the day before ovulation. Positive feedback influence of this peak value is considered essential for occurrence of the midcycle luteinizing hormone (hLH) peak and consequently, for ovulation. During pregnancy the E2 concentration increases considerably and remains high throughout pregnancy.

This assay of E2 is a valuable tool for assessing and researching the etiology of amenorrhea and/or infertility in female samples. In male samples, serum E2 measurements are used for researching feminizing syndromes.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences, Inc. Estradiol Saliva ELISA Kit, is based on the competition principle and microplate separation. An unknown amount of estradiol present in a saliva sample and a fixed amount of estradiol conjugated to horse radish peroxidase (E2-HRP) compete for binding sites with a rabbit monoclonal estradiol antiserum bound to GARGG (goat anti-rabbit gamma globulin) coated wells of a microplate. After incubation, unbound components are washed away. Enzyme substrate solution is then added, and a blue color formed. This reaction is stopped with an acid solution to produce a yellow color. The optical density is then read at 450 nm. The amount of E2-HRP detected is inversely proportional to the amount of estradiol in a sample.

PROCEDURAL CAUTIONS AND WARNINGS

1. 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.
2. Control materials or saliva pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.

9. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
10. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
11. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
12. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

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 non- reactive 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

Collection: This sample collection and processing procedure must be followed

- Avoid food consumption, drinking coffee or alcohol, smoking, or chewing gum on (1) hour prior to sample collection.
- Rinse mouth thoroughly with water 15 minutes prior to collection
- Collect whole saliva by unstimulated passive drool by allowing saliva to drip off the lower lip into a graduated plastic test tube, or by allowing saliva to accumulate in the floor of the mouth and spitting it into the recommended collection device.
- Time and date specimen. Refrigerate then freeze (-20C or below) samples until day of assay. On day of assay, thaw samples to facilitate precipitation of mucins. Centrifuge at 1500g for ten minutes. Bring samples to room temperature and assay.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

SAMPLE STABILITY

Storage	Room Temperature 20-30C	37C	2-8C	<-15C (7 freeze/thaw cycles)	<-15C (long term)
Stability	Up to 7 days	Up to 7 days	Up to 7 days	Up to 7 days	Up to 9 months

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µL
2. Disposable pipette tips

3. Distilled or deionized water
4. Microplate or orbital shaker
5. Vortex mixer
6. Plate sealers
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. GARGG Plate — Ready To Use
   - Contents: One 96-well (12x8) goat anti-rabbit gamma globulin (GARGG) microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. Stock Estradiol (17βE2) in BSA Buffer Calibrator–Concentrated
   - Contents: 1 bottle 3200 pg/mL Estradiol
   - Volume: 0.250 mL
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months 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.

   Preparation of conjugate working solution: from stock 3200 pg/mL, dilute 1:100 with assay buffer to obtain the 32 pg/mL calibrator. Make serial dilutions (in assay buffer) starting with the 32 pg/mL calibrators to obtain the following calibrator concentrations: 16 pg/mL, 8 pg/mL, 4 pg/mL, 2 pg/mL, 1 pg/mL. “0” calibrator is assay buffer

3. Assay Buffer — Ready To Use
   - Contents: 1 bottle
   - Volume: 20 mL
   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months or as indicated on label

4. Controls — Ready To Use
   - Contents: Two vials containing salivary estradiol. Concentration is located on vial
   - Volume: 1.0 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label

5. Salivary Estradiol Antibody – Ready to Use
   - Contents: 1 Bottle (blue solution)
   - Volume: 3 mL
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

6. Salivary Estradiol Horse Radish Peroxidase Conjugate Concentration
   - Contents: 1 amber bottle with yellow solution
   - Volume: 0.200 mL/bottle

Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. E2-HRP Conjugate Buffer – Ready to Use
TO BE USED WITH WORKING REAGENT PREPERATION ONLY
Contents: One vial
Volume: 4 mL
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label. Once prepared, use within 3 weeks
Preparation: Determine the amount of working E2-HRP working reagent needed and dilute 1:20 with conjugate buffer. For example, mix 125 µL (0.125 mL) of E2-HRP concentrate to a total volume of 2.5 mL with conjugate buffer. This is sufficient for 100 wells.

8. Wash solution – 10x concentrated
Contents: One bottle
Volume: 50 mL
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 with deionized water

9. Color Development Reagent – Ready to Use
Contents: One amber bottle containing TMB plus hydrogen peroxide.
Volume: 15 mL
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

10. Stopping Solution – Ready to Use
Contents: One bottle
Volume: 15 mL
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Important Notes:

- All reagents must reach room temperature before use.
- Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
- The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.
- It is recommended that the calibrators, controls and samples should be tested in duplicate and the mean value should be used to report results.

1. Prepare working solutions of the conjugate, wash buffer and standards (refer to reagents provided and preparation section).
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.

3. To the GARGG microplate dispense 100 µL of the previously prepared working Estradiol Calibrators (0, 1, 2, 4, 8, 16 and 32 pg/mL), controls, and saliva samples. Mix the microplate by shaking gently (manual) for a few seconds.
4. Add 25 µL of Estradiol HRP Working Reagent.
5. Add 25 µL of Anti-Estradiol EIA Antibody.
6. Cover the microplate with plastic sealer. Incubate by shaking on a microplate orbital shaker set at 500 – 900 rpm for 2 hours at room temperature.
7. After incubation decant content of the wells. Wash 3 times with 300 µL of diluted Wash Solution. After the 3rd wash invert GARGG microplate on absorbent paper and tap dry.
8. Dispense 125 µL of Color Development reagent into each well. Shake briefly (manual). Cover microplate with plastic sealer. Incubate for 30 minutes at room temperature.
9. Dispense 125µL of Stopping Solution into each microtiter well of the GARGG plate. Shake briefly (manual). Color changes from blue to yellow.
10. Read at 450 nm on a microplate reader within 10 minutes.
11. Note: If samples exceed the highest calibrator, dilute with zero calibrator and make appropriate concentration correction.

CALCULATIONS

1. Compute the average optical density (OD) for the zero (Bo) calibrator.
2. Calculate the percent bound (B/Bo) for each calibrator, control and unknown by dividing the average OD (B) by the average OD for the zero (Bo) x 100.
3. Plot percent bound (B/Bo) versus the calibrator concentrations and draw the best fit for the curve.
4. Plot percent bound (B/Bo) of the controls and unknowns to determine saliva estradiol concentrations.
5. Alternatively determine the concentrations of the controls and unknowns by interpolation using software capable of logistics using a 4-parmeter sigmoid minus curve fit.

Analytical measuring range (AMR) – 1 pg/mL-32 pg/mL

Samples with estradiol values greater than 32 pg/mL should be diluted 1:10 with zero (0) calibrator and re-run for accuracy. Obtain the final estradiol concentration by multiplying the diluted sampled by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator (pg/mL)	Mean Absorbance (450 nm)	% B/Bo	Value (pg/mL)
0	2.16	100.0	0
1	1.82	84.3	1
2	1.65	76.4	2
4	1.33	61.6	4
8	0.86	39.8	8
16	0.50	23.1	16
32	0.32	14.8	32
Control #1	1.53	70.8	2.5

Control #2	0.73	33.8	10.7
Sample #1	1.65	76.4	1.9
Sample #3	0.97	44.9	6.9

QUALITY CONTROL

The expected values for the controls are stated on the label of each control which are included in the kit. The results can only be accepted if the expected values are met.

EXPECTED VALUES

The following AM values were obtained from apparently healthy subjects with the Eagle Biosciences Direct Estradiol Saliva ELISA Kit, Cat #EST31-K01

Estradiol (pg/mL)		**Median**	**Range**
Females Premenopausal (not using contraceptives) n=90, Age 21- 50 yrs	Follicular Phase	3.16	2.13-5.6
	Luteal Phase	3.15	1.42-14.43
Females Postmenopausal n=51, Age 51 – 75 yrs		2.53	0.80-4.07
Males n=43, Age 20 – 70 yrs		2.68	1.07-5.00

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity

C-18 Steroids	**Spiked Concentration**	**% Cross Reactivity**
Estradiol-17ß	10,000 pg/mL	100.00
Estradiol-17a	10,000 pg/mL	0.0112
Estriol	10,000 pg/mL	0.0615
Estrone	10,000 pg/mL	0.9225
Estrone-3-SO4	10,000 pg/mL	0.3398
Estriol-3-SO4	10,000 pg/mL	0.0066
C-19 Steroids		
Androstenedione	10,000 pg/mL	0.0008
Testosterone	10,000 pg/mL	0.0082
5a-Dihydrotestosterone	10,000 pg/mL	0.0050
Dehydroepiandrosterone SO4	10,000 pg/mL	0.0030
Androstenedione	10,000 pg/mL	0.0148
C-21 Steroids		
Progesterone	10,000 pg/mL	<0.001
17-OH Progesterone	10,000 pg/mL	0.0048
Pregnenolone	10,000 pg/mL	0.0044
17-OH-Pregnenolone	10,000 pg/mL	0.0096
Deoxycorticosterone	10,000 pg/mL	0.0063
11-Desoxycortisol	10,000 pg/mL	0.0085
Corticosterone	10,000 pg/mL	0.0171
Aldosterone	10,000 pg/mL	0.0072
Cortisol	10,000 pg/mL	0.0104

DETECTION LIMITS

The LOB (limit of the blank) and the LOD (limit of detection) were determined by generating one hundred twenty (120) measurements each of "estradiol free salvia" and low level (<1 pg/mL) estradiol samples (Reference, CLSI EP 17-A, protocols for Determination of Limits of Detection and Limits of Quantitation).

Limit of the Blank (LoB) pg/mL	Limit of Detection (LoD) pg/mL
0.3680	0.5239

INTRA-ASSAY PRECISION

The intra-assay precision was determined from the mean of 20 replicates of low, medium and high samples.

Sample	N	Mean	SD	CV %
Low	20	3.29	0.352	10.7
Medium	20	27.24	0.999	3.7
High	20	52.56	3.103	5.9

INTER-ASSAY PRECISION

The inter-assay precision was determined from the mean of the average duplicates of 12 separate assays with low, medium and high samples

Sample	N	Mean	SD	CV %
Low	12	3.01	0.266	8.8
Medium	12	26.13	0.939	3.6
High	12	49.19	4.552	9.3

REPEATABILITY

This study was conducted during 5 days of a familiarization period and 20 days of testing. Two assays were performed daily. Three (3) different reagent lots and three (3) saliva pools were used for the study (low, medium and high). The pools were aliquoted and frozen until day of assay.

PRECISION LOW CONCENTRATION POOL

	SD	CV %
Within Run	0.047	1.7
Between Run	0.052	1.9
Repeatability	0.105	3.9
Total Device Precision	0.126	4.6

PRECISION MEDIUM CONCENTRATION POOL

	SD	CV %
Within Run	0.323	1.2
Between Run	0.096	0.4
Repeatability	0.552	2.1
Total Device Precision	0.647	2.5

PRECISION HIGH CONCENTRATION POOL

	SD	CV %
Within Run	0.321	0.7
Between Run	0.964	2.1
Repeatability	0.859	1.8
Total Device Precision	1.331	2.8

LINEARITY

S=10 samples (dilutions)

Concentration=(C1*V1+C10*V10)/(V1+V10)

	C1 (pg/mL)	V1 (mL)	C10 (pg/mL)	V10 (mL)	Calculated Concentration (pg/mL)	Obtained Concentration (pg/mL)	Recovery (%)
1				*	0.40	0.42	105
2	0.42	0.889	81.57	0.111	9.43	8.44	90
3	0.42	0.778	81.57	0.222	18.44	16.61	90
4	0.42	0.667	81.57	0.333	27.44	24.88	91
5	0.42	0.556	81.57	0.444	36.45	36.32	100
6	0.42	0.444	81.57	0.556	45.54	45.50	100
7	0.42	0.333	81.57	0.667	54.55	56.08	103
8	0.42	0.222	81.57	0.778	63.55	65.69	103
9	0.42	0.111	81.57	0.889	72.56	76.66	106
10				*	80.00	81.57	102

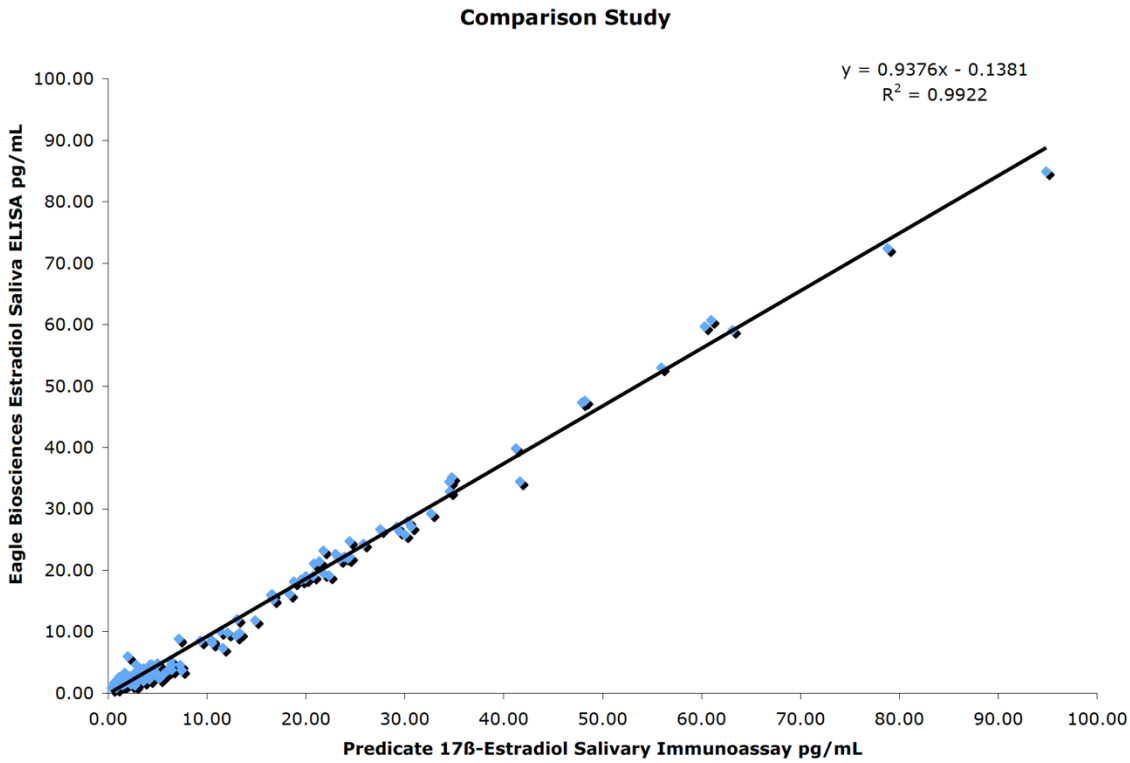
RECOVERY

Seven (7) saliva samples containing different levels of endogenous estradiol were spiked with known quantities of estradiol, assayed and the percent recovery determined.

Sample	Endogenous (pg/mL)	Estradiol Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1	1.00	64.0	65.00	60.37	92.9
2	3.20	32.0	35.20	34.53	98.1
3	4.42	16.0	20.42	19.55	95.7
4	4.24	16.0	12.24	11.53	94.2
5	24.61	4.0	28.61	26.82	93.7
6	40.90	2.0	42.90	42.02	97.9
7	4.86	1.0	5.86	5.30	90.4

COMPARISON STUDY

One hundred twenty-five (125) saliva samples spanning the analytical measuring range (AMR) and beyond were analyzed by comparing the Eagle Biosciences Direct Estradiol Saliva ELISA Kit, Cat #EST31-K01 with a commercially available predicate Estradiol salivary Immunoassay. See correlation results below.



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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.