Estriol Saliva LIA Assay Kit

Catalog Number:
ESL32-L01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences Estriol Saliva LIA Kit is for the direct quantitative determination of estriol in human saliva by a chemiluminescence immunoassay (LIA). The Eagle Biosciences Estriol Saliva LIA kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

During pregnancy the determination of estriol is used for monitoring fetal well being. Therefore, during pregnancy the estriol level can detect abnormalities in the development of the fetus. As pregnancy progresses in time the concentration of Estriol also increases. The unconjugated estriol saliva determination is of great clinical importance to follow the progress of pregnancy since the level of salivary unconjugated estriol reflects the unconjugated estriol concentration in serum, which reflects the biologically active fraction. The collection of saliva has advantages compared to urine and serum since it is a non-invasive procedure which can be easily collected at home and stored frozen. Due to the low concentration of unconjugated estriol in saliva we have developed an assay system using a chemiluminescent immunoassay (LIA). The assay is very sensitive (0.03 ng/mL), precise and accurate along with a short incubation time of 75 minutes at RT° on a shaker.

PRINCIPLE OF THE ASSAY

The principle of the following chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, control 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 luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of estriol in the sample. A set of calibrators are used to plot a standard curve from which the amount of Estriol in patient samples and controls can be directly read.

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 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. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. 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.
13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the determination of estriol in human saliva. The kit is not calibrated for the determination of estriol 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 intended for research use only and should not be used in diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human fluids that may be used in the preparation of the standards and control have been tested and found to be non-reactive for Hepatitis B surface antigen and have 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 human 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 (Salivette by Sarstedt may be used) 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.

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 20, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate luminometer.

REAGENTS PROVIDED

1. Rabbit Anti-Estriol 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. Estriol-Biotin Conjugate Concentrate — Requires Preparation X200
   Contents: Estriol-biotin conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation of conjugate working solution: Dilute biotin conjugate concentrate 1:200 in assay buffer B before use (example: 10 µL of biotin conjugate concentrate in 2 mL of assay buffer B). If the whole plate is to be used dilute 40 µL of biotin conjugate concentrate in 8 mL of assay buffer B. Discard any that is left over.

3. Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate – Requires Preparation x100
   Contents: Avidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation of conjugate working solution: Dilute HRP conjugate concentrate 1:100 in assay buffer A before use (example: 20 µL of HRP conjugate concentrate in 2 mL of assay buffer A). If the whole plate is to be used dilute 120 µL of HRP conjugate concentrate in 12 mL of assay buffer A. Discard any that is left over.

4. DHEAS Calibrators — Ready To Use
   Contents: Eight vials containing estriol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estriol.
   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.03 ng/mL	0.7 mL
Calibrator C	0.1 ng/mL	0.7 mL
Calibrator D	0.3 ng/mL	0.7 mL
Calibrator E	1 ng/mL	0.7 mL
Calibrator F	3 ng/mL	0.7 mL
Calibrator G	10 ng/mL	0.7 mL
Calibrator H	30 ng/mL	0.7 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

5. Controls — Ready To Use
   - Contents: Two vials containing estriol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estriol. Refer to vial label for expected value and acceptable range.
   - Volume: 0.7 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.

6. 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 one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

7. Assay Buffer A – Avidin-HRP Diluent – Ready to Use
   - Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   - Volume: 20 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

8. Assay Buffer B – Estriol-Biotin Diluent – Ready to Use
   - Contents: One bottle containing a 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.

9. LIA Substrate Reagent A – Requires Preparation
   - Contents: One vial containing luminol enhancer.
   - Volume: 1 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: See preparation of LIA working substrate solution.

10. LIA Substrate Reagent B – Requires Preparation
    - Contents: One vial containing peroxide solution.
    - Volume: 2 mL/vial
    - Storage: Refrigerate at 2–8°C
    - Stability: 12 months or as indicated on label.
    - Preparation: See preparation of LIA working substrate solution.

11. LIA Substrate Reagent C – Requires Preparation

Contents:	One bottle containing buffer with a non-mercury preservative.
Volume:	15 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.
Preparation:	See preparation of LIA working substrate solution.

PREPARATION OF LIA WORKING SUBSTRATE SOLUTION

Mix 1 part of LIA substrate reagent A with 2 parts of LIA substrate reagent B and dilute this mixture 1:3.33 with LIA substrate reagent C. This gives the ready to use substrate solution. Prepare fresh for each use.
If the whole plate is to be used prepare working substrate solution as follows:
Combine 1 mL of LIA substrate reagent A with 2 mL of LIA substrate reagent B. To the 3 mL of this mixture add 10 mL of LIA substrate reagent C.
Total volume = 13 mL of working substrate solution.
Stability: Working substrate solution is stable for 24 hours at room temperature.

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.

1. Prepare working solutions of both conjugates, wash buffer and LIA substrate (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. Pipette 50 µL of each calibrator, control and pretreated specimen sample (refer to specimen pretreatment section) into correspondingly labelled wells in duplicate.
4. Pipette 50 µL of the estriol-biotin conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 45 minutes at room temperature.
6. Wash the wells 5 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 100 µ L of the avidin-HRP conjugate working solution into each well. (We recommend using a multichannel pipette.)
8. Incubate on a plate shaker (approximately 200 rpm) for 20 minutes at room temperature.
9. Wash the wells 5 times with 300 µL of diluted wash buffer per well in the same manner as step 6.
10. Pipette 100 µL of LIA working substrate solution into each well. (We recommend using a multichannel pipette.)
11. Shake for 5 seconds. Incubate for 10–30 minutes at room temperature without shaking.
12. Measure the RLUs in each well on a microplate luminometer.

CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 30 ng/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.

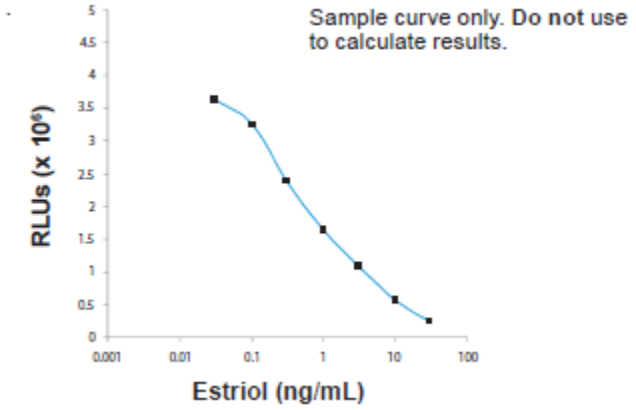
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLU_{MAX} (%)
A, 0 ng/mL	4649	4221	4435	100
B, 0.03 ng/mL	3763	3536	3650	82.3
C, 0.1 ng/mL	3313	3193	3253	73.3
D, 0.3 ng/mL	2441	2357	2399	54.1
E, 1 ng/mL	1671	1645	1658	37.4
F, 3 ng/mL	1114	1053	1084	24.4
G, 10 ng/mL	593	555	574	12.9
H, 30 ng/mL	256	260	258	5.8

**It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the estriol LIA kit is **0.03 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the estriol LIA kit with estriol cross-reacting at 100%:

Steroid	% Cross Reactivity
Estriol	100
Estriol 3-D-Glucuronide	0.15
Estrone	0.10
Estrone 3-SO_4	ND
Estrone 3-D-Glucuronide	ND
Estradiol	0.51
Estradiol 3-SO_4	0.01
Estradiol 17-D-Glucuronide	0.02
Estradiol 3-Glucuronide	0.03

No cross-reaction was detected with DHEAS, Diethylstilbesterone, Equilin and Progesterone.

INTRA-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	5.87	0.58	9.9
2	36.75	2.88	7.84
3	89.21	5.68	6.37

INTER-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	4.38	0.32	7.2
2	25.41	1.63	6.4
3	43.31	2.55	5.9

RECOVERY

Samples were spiked by adding different estriol standards (1:1 volume/volume) to three patient saliva samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	3.44	-	-
+ 1	2.37	2.22	106.7
+ 3	2.94	3.22	91.3
+ 10	6.37	6.72	94.8
2 Unspiked	5.49	-	-
+ 1	2.94	3.25	90.5
+ 10	7.71	7.75	99.5
+ 100	47.50	52.75	90.0
3 Unspiked	29.4	-	-
+ 0.3	16.17	14.65	110.4
+ 1	17.75	15.2	116.9
+ 3	19.26	16.2	118.9

LINEARITY

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

Sample	Obs. Result	Exp. Result	Recovery %
1	10.22	-	-
1:2	3.73	5.11	73.0
1:4	1.96	2.56	76.6
1:8	0.97	1.23	78.9
2	16.55	-	-
1:2	9.05	8.28	102.8
1:4	50.6	4.14	122.2
1:8	2.07	2.07	100.0
3	29.55	-	-
1:2	13.8	14.78	93.4
1:4	5.98	7.39	79.8
1:8	3.18	3.20	106.0

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