Estrone LIA

For the quantitative determination of estrone in human serum.

Please read carefully due to Critical Changes, e.g., Assay Procedure (step 8)

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	11-ESRHU-L01
Size:	96 wells
Version:	4 - August 09, 2013 - ALPCO August 29, 2013

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

INTENDED USE

For the direct quantitative determination of estrone in human serum by a chemiluminescence immunoassay (LIA). For Research Use Only. Not for Use in Diagnostic Procedures.

PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. 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 estrone in the sample. A set of calibrators are used to plot a standard curve from which the amount of estrone in samples and controls can be directly read.

APPLICATIONS

Estrone is a steroid like estriol and estradiol, belonging to the class of estrogens. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized the main action of the estrogens is on the growth and function of the reproductive tract in order to prepare it for the fertilized ovum.
During the follicular phase of the menstrual cycle the estrone level shows a slight increase. The production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases.

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 serum 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 kit 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, 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 estrone in human serum. The kit is not calibrated for the determination of estrone in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for diagnosis. For example, the occurrence of heterophilic antibodies in subjects regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the diagnosis should include all aspects of a subject's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum 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. However no test method 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 direct contact with reagents. In case of contact, wash with plenty of water.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4^{o}C for up to 24 hours or at -10^{o}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

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

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150, 300 µL and 1000 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate luminometer

REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-Estrone Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months or as indicated on label.

2. Estrone-Biotin Conjugate Concentrate - Requires Preparation.

Contents: Estrone-biotin conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.2 mL/vial
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months or as indicated on label.
Preparation: See preparation of conjugate working solution.

3. Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: Avidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.2 mL/vial
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months or as indicated on label.
Preparation: See preparation of conjugate working solution.

Preparation of Conjugate Working Solution

Dilute both the estrone-biotin and avidin-HRP conjugate concentrates 1:100 into the same solution of assay buffer and mix thoroughly (example: To a tube containing 2 mL of assay buffer add 20 µL of estrone-biotin and 20 µL of avidin-HRP conjugate concentrates). If the whole plate is to be used add 120 µL of estrone-biotin and 120 µL of avidin-HRP conjugate concentrates to 12 mL of assay buffer. Discard any that is left over.

It is essential that the conjugate working solution be prepared, mixed and allowed to stand for at least 15 minutes prior to use. Failure to do so may result in low optical densities and increased serum values.

4. Estrone Calibrators - Ready To Use.
Contents: Six vials containing estrone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of estrone.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/mL	2.0 mL
Calibrator B	15 pg/mL	0.5 mL
Calibrator C	50 pg/mL	0.5 mL
Calibrator D	200 pg/mL	0.5 mL
Calibrator E	800 pg/mL	0.5 mL
Calibrator F	2000 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.

5. Controls - Ready To Use.
Contents: Two vials containing estrone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of estrone. Refer to vial label for 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 control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

6. Wash Buffer Concentrate - Requires Preparation.
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 of wash buffer working solution: Dilute wash buffer concentrate 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of wash buffer concentrate in 450 mL of water.

7. Assay Buffer - 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.

8. LIA Substrate Reagent A - Requires Preparation.
Contents: One vial containing luminol enhancer.
Volume: 1.5 mL/vial
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See preparation of LIA working substrate solution.

9. LIA Substrate Reagent B - Requires Preparation.
Contents: One vial containing peroxide solution.
Volume: 1.5 mL/vial
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See preparation of LIA working substrate solution.

10. LIA Substrate Reagent C - Requires Preparation.
Contents: One bottle containing buffer with a non-mercury preservative.
Volume: 16 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See preparation of LIA working substrate solution.

Preparation of LIA Working Substrate Solution
In a clean plastic container (glass is not suitable) mix 1 part of LIA substrate reagent A with 1 part of LIA substrate reagent B and10 parts of LIA substrate reagent C. This gives the ready to use substrate solution. If the whole plate is to be used prepare working substrate solution as follows: Combine 1.4 mL of LIA substrate reagent A with 1.4 mL of LIA substrate reagent B and 14 mL of LIA substrate reagent C. It is suggested to wait at least 2 minutes prior to use after preparation of the working substrate solution. The working substrate solution is stable for up to 2 hours at room temperature. Discard the leftovers.

ASSAY PROCEDURE

Important Notes:

1. All reagents must reach room temperature before use.

2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.

3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).

After conjugate working solution is prepared, it is essential that it be mixed and allowed to stand for at least 15 minutes prior to use.

2. Remove the required number of microwell 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 (The use of a multichannel pipette is recommended).

5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour 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 150 µL of LIA working substrate solution into each well (The use of a multichannel pipette is recommended).

8. Measure the RLU/second in each well on a microplate luminometer between 10-30 minutes after addition of the substrate.

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 fit 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 2000 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.

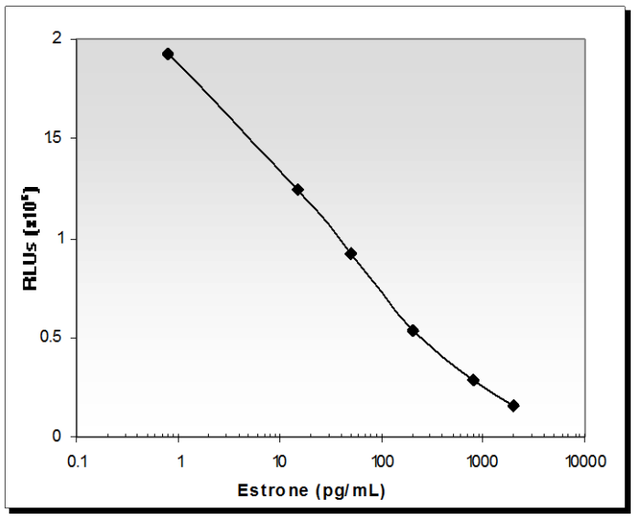
TYPICAL TABULATED DATA**

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLU_{MAX} (%)
A, 0 pg/mL	1980	1877	1928	100
B, 15 pg/mL	1256	1226	1241	64
C, 50 pg/mL	832	1004	918	48
D, 200 pg/mL	542	527	534	28
E, 800 pg/mL	311	276	294	15
F, 2000 pg/mL	169	161	165	9

**- 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.

TYPICALCALIBRATORCURVE

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 RLU of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the estrone LIA kit is
8.8 pg/mL.

SPECIFICITY (CROSS-REACTIVITY)

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

Steroid	%Cross-Reactivity
Estrone	100
Estrone-3-Sulfate	4.9
17β-Estradiol	2.2
Estrone-3-Glucuronide	1.2
17ß-Estradiol-3-Glucuronide	0.14

The following steroids were tested but cross-reacted at less than 0.1%: Androstenedione, Cholesterol, Corticosterone, Cortisol, Cortisone, DHEAS, Diethylstilbesterone, Estriol, 17ß-Estradiol-3-Glucuronide, Estradiol-Sulfate, Progesterone, 17-OH Progesterone 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	70.4	4.12	6.6
2	278.7	16.16	5.8
3	787.5	76.9	9.8

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	77.6	9.08	11.7
2	272.4	24.2	8.9
3	823.6	89.77	10.9

RECOVERY

Spiked samples were prepared by adding defined amounts of estrone to three serum samples. The results (in pg/mL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	52	-	-
+200	315	252	125
+400	557	452	120
+1000	1235	1052	117
2 Unspiked	75	-	-
+375	493	450	88.0
+750	505.23	559.81	90.3
+1500	712.44	794.88	89.6
3 Unspiked	720.11	-	-
+200	758.13	837.64	90.5
+400	856.46	955.17	89.7
+1000	1013.61	1190.24	85.1

LINEARITY

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

Sample	Obs. Result	Exp. Result	Recovery%
1	340.67	-	-
1:2	165.35	170.34	97.1
1:4	95.39	85.17	112.0
1:8	48.47	42.58	113.8
2	1086.01	-	-
1:2	508.58	543.00	93.7
1:4	232.11	271.50	85.5
1:8	114.95	135.75	84.7
3	1313.21	-	-
1:2	612.98	656.61	93.4
1:4	318.63	328.30	97.1
1:8	134.98	164.15	82.2

COMPARATIVE STUDIES

The estrone LIA kit (y) was compared with another estrone ELISA kit (x). The comparison of 50 serum samples yielded the following linear regression results:

$$y=0.8872X - 2.382,\ r^2=0.99$$

EXPECTED NORMAL VALUES

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

REFERENCES

1. Hauptmann. H. et al: Concepts for the synthesis of biotinylated steroids. Part 2: 17-estradiol derivatives as immunochemical probes. Bioconjugate Chem. 11(2000) 537-548.
2. Dressendorfer R.A. et al: Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J. Steroid Biochem. Molec.Biol.: 43 (1992) 683-692.
3. Mayer H.H.D. et al: Immunoaffinity chromatography and a biotin-streptavidin amplified immunoassay for sensitive and specific estimation of estradiol-17. J. Steroid Biochem. 35(1990) 263-269.
4. Folan J. et al: Solid-phase enzymoimmunoassay of estrone in serum. Clin. Chem. 34(1988) 1843-1846.
5. Folan J. et al: Solid-phase enzymoimmunoassay of estrone insaliva. Clin. Chem. 35:4(1989)569-572.
6. Speroff L. et al: Hormone biosynthesis, metabolism, and mechanism of action. In: Clinical gynaecologic endocrinology & infertility, 3rd ed. Williams & Wilkins. 1983:1-41.
7. Kim M.H. et al: Plasma levels of estrogen, androgens and progesterone during normal and dexamethasone treated cycles. J. Clin. Endocrinol. Metab. 39 (1974)706-712.
8. Speight A.C. et al; Non-protein bound oestrogens in plasma and urinary excretion of unconjugated oestrogens in non-pregnant women. J. Endocrino. 83(1979) 385-391.
9. Kricka, L.J., Human anti-animal antibody interferences in immunological assays. Clin. Chemistry 45:7, 1999.
10. Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol Obstet Invest 40:139-140, 1995.