DRG

DRG® Estriol CLIA (Saliva) (CLA-4653)

CE

USA: RUO

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1 INTENDED USE

For determination of Estriol in human saliva by chemiluminescence immunoassay (CLIA). In the United States, this kit is intended for Research Use Only.

2 PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (CLIA) test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and donor 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 estriol in the sample. A set of Standards are used to plot a standard curve from which the amount of Estriol in donor samples and controls can be directly read.

3 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 Standard curve must be established for every run.
7. The kit control 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 control 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. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. When dispensing the substrate, do not use pipettes in which this liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. 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.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

4 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct 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 Standard A may be used to dilute any high saliva samples. The use of any other reagent may lead to false results.

5 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. 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.

6 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 (Sali-Tubes (SLV-4158) or 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.

6.1 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.

7 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. Microwell plate luminometer

8 REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-Estriol 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°C
Stability: 12 months or as indicated on label.

2. Estriol-Biotin Conjugate Concentrate - Requires Preparation.
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: Dilute 1:100 in assay buffer B before use. Discard any unused 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: 0.3 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:100 in assay buffer A before use (example: 20µl of HRP Conjugate Concentrate in 2 ml of Assay Buffer A). Discard any unused solution.

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

Standard	Concentration	Volume/Vial
Standard A	0 ng/mL	2.0 mL
Standard B	0.03 ng/mL	0.7 mL
Standard C	0.1 ng/mL	0.7 mL
Standard D	0.3 ng/mL	0.7 mL
Standard E	1 ng/mL	0.7 mL
Standard F	3 ng/mL	0.7 mL
Standard G	10 ng/mL	0.7 mL
Standard 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 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: 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.

7. Assay Buffer A - Avidin-HRP Diluent - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 20 ml/vial
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 vial containing a protein-based buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

9. CLIA Substrate Reagent A - Requires Preparation.
Contents: One bottle containing luminol enhancer.
Volume: 1 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below.

10. CLIA 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 below.

11. CLIA Substrate Reagent C - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below.

8.1 Preparation of Working Substrate Solution:

Mix **1 part** of the chemiluminescence **substrate reagent A** with **2 part of substrate reagent B** and dilute this mixture **1:3.33 with 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 reagent A with 2 ml of reagent B. To the 3 ml of this mixture add 10 ml of reagent C.
Total volume=13 ml of working substrate solution.

Stability: Working substrate solution is stable for 24 hours at room temperature.

9 ASSAY PROCEDURE

Specimen Pretreatment:

See under specimen pre-treatment

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.

Procedure:

1. Prepare working solutions of both conjugates, wash buffer and CLIA substrate (refer to reagents provided and preparation section).
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 Standard, control and specimen sample 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.

7. Pipette 100 µl 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 5.
10. Pipette 100 µl of chemiluminescence working substrate solution into each well (We recommend using a multichannel pipette).
11. Shake for 5 seconds. Incubate for 10-15 minutes at room temperature without shaking.
12. Measure the RLUs in each well on a microplate luminometer within 20 minutes.

10 CALCULATIONS

1. Calculate the mean RLU of each Standard duplicate.
2. Draw a Standard curve on semi-log paper with the mean RLUs on the Y-axis and the Standard 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 Standard curve.
5. If a sample reads more than 100 ng/ml then dilute it with Standard A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA**

Standard	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLUMAX (%)
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	5.5

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

11 REFERENCES

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