DRG® Estrone Saliva (CLA-4655)

CE

RUO in the USA

Revised 7 May 2009 (Vers. 2.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

For determination of estrone in human saliva by a chemiluminescence immunoassay (CLIA).

2 PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (CLIA) test follows a two-step competitive binding scenario.

Competition occurs between an unlabeled antigen (present in standards, control and samples) and a biotin-labelled antigen for a limited number of anti-estrone antibody binding sites on the microwell plate. After washing the streptavidin-horseradish peroxidase conjugate is incubated and forms a complex with the bound biotinylated estrone. The washing and decanting procedures remove unbound materials, and then 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 is used to plot a standard curve from which the amount of estrone in saliva 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 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 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. 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.

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13. 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 determination of estrone in human saliva. The kit is not calibrated for the determination of estrone 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.

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 2-3 mL of saliva into a clean glass tube without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection and wait a few minutes to start. 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.

7 SPECIMEN PRETREATMENT

1. Specimen samples are to be centrifuged. The supernatants are to be transferred into clean tubes.
2. The tubes containing the supernatant are to be placed in a water bath and heated at 60-70°C for 1 hour,
3. Allow heated samples to reach room temperature before assaying.

8 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

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

9 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°C - 8°C
Stability: 12 months or as indicated on label.

2. Estrone-Biotin Conjugate Concentrate - X50

Contents: Estrone-biotin conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.3 mL/vial
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.

Preparation of conjugate working solution: Dilute biotin conjugate concentrate 1:50 in biotin conjugate buffer before use (example: 20 µL of biotin conjugate concentrate in 1 mL of biotin conjugate buffer). If the whole plate is to be used dilute 240 µL of biotin conjugate concentrate in 12 mL of biotin conjugate buffer. Discard any that is left over.

3. Streptavidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate - X50

Contents: Streptavidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.4 mL/vial

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

Preparation of conjugate working solution: Dilute HRP conjugate concentrate 1:50 in HRP conjugate buffer before use (example: 20 µL of HRP conjugate concentrate in 1 mL of HRP conjugate buffer). If the whole plate is to be used dilute 320 µL of HRP conjugate concentrate in 16 mL of HRP conjugate buffer. Discard any that is left over.

4. Estrone Saliva Standards - Ready To Use.

Contents: Six vials containing estrone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a precise quantity of estrone.

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

Standard	Concentration	Volume/Vial
Standard A	0 pg/mL	4.0 mL
Standard B	2 pg/mL	1.0 mL
Standard C	10 pg/mL	1.0 mL
Standard D	50 pg/mL	1.0 mL
Standard E	200 pg/mL	1.0 mL
Standard F	800 pg/mL	1.0 mL

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

5. Control - Ready To Use.

Contents: One vial containing estrone in a protein-based buffer with a non-mercury preservative. Refer to vial label for expected value and acceptable range.
Volume: 1 mL/vial
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.

6. Biotin Conjugate Buffer - Ready To Use.

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 13 mL/bottle
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.

7. HRP Conjugate Buffer - Ready To Use.

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 20 mL/bottle
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.

8. Wash Buffer Concentrate - X10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2°C - 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.

9. Rinse Solution - Ready To Use.

Contents: One bottles containing buffer with a non-mercury preservative.
Volume: 60 mL/bottle
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.

10. CLIA Substrate Reagent A - Requires Preparation.

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

11. CLIA Substrate Reagent B - Requires Preparation.

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

12. CLIA Substrate Reagent C - Requires Preparation.

Contents: One bottle containing buffer with a non-mercury preservative.
Volume: 16 mL/bottle
Storage: Refrigerate at 2°C - 8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of CLIA working substrate solution.

Preparation of CLIA Working Substrate Solution

In a clean container mix 1 part of CLIA substrate reagent A with 2 part of CLIA substrate reagent B and 20 parts of CLIA 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 0.7 mL of CLIA substrate reagent A with 1.4 mL of CLIA substrate reagent B and 14 mL of CLIA 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 8 hours at room temperature. Discard the leftovers.

10 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.
4. Prepare working solutions of both conjugates, wash buffer and CLIA substrate (refer to reagents provided and preparation section).
5. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
6. Pipette 100 µL of each standard, control and pretreated specimen sample (refer to specimen pretreatment section) into correspondingly labelled wells in duplicate.
7. Pipette 100 µL of the estrone-biotin conjugate working solution into each well (We recommend using a multichannel pipette).
8. Cover the plate and incubate for 1 hour on a plate shaker (approximately 200 rpm) at room temperature.
9. Wash the wells 5 times with 300 µL of wash buffer working solution per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
10. Pipette 150 µL of the streptavidin-HRP conjugate working solution into each well (We recommend using a multichannel pipette).
11. Cover the plate and incubate for 30 minutes on a plate shaker (approximately 200 rpm) at room temperature.
12. Wash the wells 5 times in the same manner as step 6.
13. Rinse the wells 1 times with 300 µL of rinse solution per well and tap the plate against absorbent paper to ensure it is dry
14. Pipette 150 µL of CLIA working substrate solution into each well (We recommend using a multichannel pipette).
15. Measure the RLU/second in each well on a microplate luminometer within 5-20 minutes after addition of the substrate.

11 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 800 pg/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.

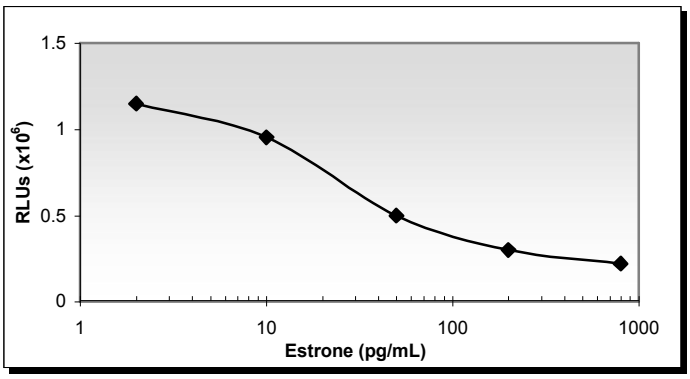
TYPICAL TABULATED DATA**

Standard	RLU 1	RLU 2	Mean RLU	RLU/RLU$_{MAX}$ (%)
A, 0 pg/mL	134500	131500	133000	100
B, 2 pg/mL	119600	110500	115000	86
C, 10 pg/mL	86500	84600	85500	64
D, 50 pg/mL	49000	47600	48300	36
E, 200 pg/mL	20600	19700	20100	15
F, 800 pg/mL	8700	8300	8500	6

**- 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

Sample curve only. **Do not** use to calculate results.



11.1 REFERENCES

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