DRG® DHEA-S CLIA (CLA-4652)

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1 INTENDED USE

For the direct quantitative determination of DHEAS in human serum by chemiluminescence immunoassay (CLIA). For in vitro 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 patient 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 DHEAS in the sample. A set of calibrators are used to plot a standard curve from which the amount of DHEAS in patient samples and controls can be directly read.

3 CLINICAL APPLICATIONS

DHEAS is one the most abundant circulating steroid. It is produced by the adrenal and gonads. As a result, the determination of the level of DHEAS in serum is important in the evaluation of the functional state of these glands. It is also a precursor of testosterone and estrone. Besides the adrenals, in females, the ovaries have been shown to be an important source of DHEAS.

It has been reported that there is a fluctuation day by day of DHEAS in women during the ovulatory cycle.

The principal production of testosterone in females is from the conversion of other related androgens especially DHEAS. An abnormal testosterone levels in women should be accompanied by the estimation of serum DHEAS. The use of serum testosterone determination in conjunction with Elisa DHEAS can be used to determine if the source of excess androgen production is ovarian or adrenal.

4 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 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 these 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.

5 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of DHEAS in human serum. The kit is not calibrated for the determination of DHEAS 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. Only the urine diluent may be used to dilute any high urine samples. The use of any other reagents may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6 SAFETY CAUTIONS AND WARNINGS

Human serum that may be used in the preparation of the standards and control 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. 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 blood specimen.

7 SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 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°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

8 CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

9 SPECIMEN PRETREATMENT

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

10 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

11 REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-DHEAS 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. DHEAS-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: DHEAS-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 300 µl/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12ml of assay buffer. Discard any that is left over.

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

Calibrator	Concentration	Volume/Vial
Calibrator A	0 µg/dL	2.0 mL
Calibrator B	0.02 µg/dL	0.5 mL
Calibrator C	0.1 µg/dL	0.5 mL
Calibrator D	0.5 µg/dL	0.5 mL
Calibrator E	2.5 µg/dL	0.5 mL
Calibrator F	10 µg/dL	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.

4. Control - Ready To Use.
Contents: One vial containing DHEAS in a protein serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of DHEAS. Refer to vial label for expected value and 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.

5. 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.

6. Assay Buffer - 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.

7. Cheminluminescence 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.

8. Cheminluminescence Substrate Reagent B - Requires Preparation.
Contents: One vial containing peroxide solution.

Volume: 1 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below.

9. Cheminluminescence 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.

Preparation of Working Substrate Solution:

Mix 1 part of the chemiluminescence substrate reagent A with 1 part of reagent B and dilute this mixture 1:5 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 1 ml of reagent B. To the 2 ml of this mixture add 10 ml of reagent C.
Total volume=12 ml of working substrate solution.

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

12 ASSAY PROCEDURE

Specimen Pretreatment: None.

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. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
2. Pipette 25 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 100 µl of the conjugate working solution into each well
   (We recommend using a multichannel pipette).
4. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
5. 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).
6. Pipette 100 µl of chemiluminescence working substrate solution into each well
   (We recommend using a multichannel pipette).
7. Incubate for 10-15 minutes at room temperature, without shaking.

8. Measure the RLUs in each well on a microplate luminometer within 20 minutes after addition of the substrate.

13 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 10 µg/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.

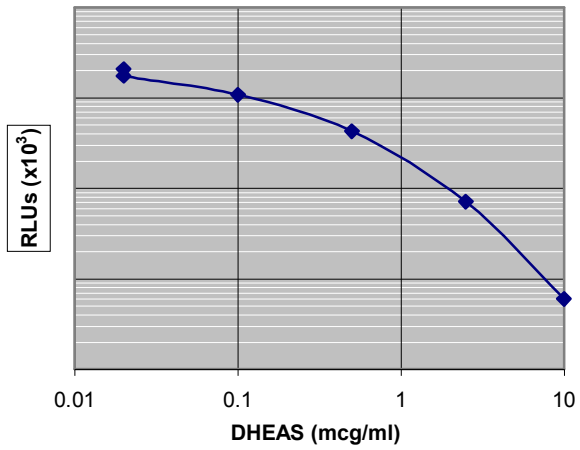
TYPICAL TABULATED DATA**

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLU_{MAX} (%)
A, 0 µg/ml	2120	2021	2070	100
B, 0.02 µg/ml	1856	1619	1737	83.9
C, 0.1 µg/ml	1076	1075	1076	51.9
D, 0.5 µg/ml	446	407	426	20.6
E, 2.5 µg/ml	72	69	71	3.4
F, 10 µg/ml	8	4	6	0.3

**- 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.



14 PERFORMANCE CHARACTERISTICS

14.1 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 DHEAS CLIA kit is **0.02** µg/ml**.**

14.2 SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct DHEAS CLIA kit with DHEAS cross-reacting at 100%.

Steroid	%Cross Reactivity
DHEAS	100
Androsterone	16.0
Testosterone	1.7
Progesterone	0.9
DeoxyDHEAS	5.6
Pednisone	5.6
Dexamethasone	1.6

No cross reaction was detected with Cortisol, Estradiol, Estrone, Estrone sulphate or pregnenolone.

14.3 INTRA-ASSAY PRECISION

Three samples were assayed sixteen times each on the same calibrator curve. The results (in µg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.7	0.03	4.4
2	2.43	0.11	4.7
3	5.84	0.25	4.2

14.4 INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of two weeks. The results (in µg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.61	0.045	7.4
2	2.37	0.16	6.7
3	6.55	0.33	5.1

14.5 RECOVERY

Samples were Spiked by adding different DHEAS standards at 1:1 volume to two patient serum samples.
The results (in µg/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	0.9	-	-
+ 0.1 µg/ml standard	0.5	0.5	100.0
+ 0.5 µg/ml standard	0.8	0.7	114.2
+ 2.5 µg/ml standard	1.9	1.7	111.8
2 Unspiked	0.77	-	-
+ 2 µg/dL standard	0.52	0.44	117.0
+ 5 µg/dL standard	1.58	1.64	96.6
+ 10 µg/dL standard	5.44	5.39	100.9

14.6 LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µg/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	2.22	-	-
1:2	1.28	1.11	115.3
1:4	0.62	0.56	111.7
1:8	0.31	0.28	111.7
2	2.30	-	-
1:2	1.16	1.15	100.9
1:4	0.55	0.58	95.7
1:8	0.29	0.29	100
3	9.44	-	-
1:2	4.48	4.72	94.9
1:4	2.34	2.36	99.2
1:8	1.17	1.18	99.2

15 EXPECTED NORMAL VALUES-SERUM

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

Group	Range (µg/ml)
Males	0.39 – 4.65
Females	0.46 – 2.75
Postmenopausal Females	0.48 – 2.08

16 REFERENCES

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