EAGLE BIOSCIENCES

Dehydroepiandrosterone Sulfate (DHEAS) ELISA Assay Kit

Catalog Number:
DHS31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2 (14 MAY 24)

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INTENDED USE

The Eagle Biosciences Dehydrospiandrosterone Sulfate (DHEAS) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of human DHEAS levels in serum. The Eagle Biosciences DHEAS ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Dehydroepiandrosterone sulfate (DHEAS) is produced by the adrenals and gonads. As a result, the determination of the level of DHEA-S in serum is important in the evaluation of the functional state of these glands. DHEAS is 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 principle production of testosterone in females is from conversion of other related androgens, especially DHEAS. An abnormal testosterone level in women should be accompanied by the estimation of serum DHEAS. The use of serum testosterone determination in conjunction with Elisa of DHEAS can be used to determine if the source of excess androgen production is ovarian or adrenal.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls 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 enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of DHEAS in the sample. A set of standards is used to plot a standard curve from which the amount of DHEAS in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves. d Wash hands thoroughly after performing the test. e Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Do not use this kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.

7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >10 µg/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other calibrator may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.

29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.

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. The use of any other reagent may lead to false results.
5. This kit is intended for research use only and should not be used as a diagnostic tool.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive 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. 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 blood 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 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.

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 25, 50, 150, 200 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Rabbit Anti-DHEAS 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. **DHEAS-Horseradish Peroxidase (HRP) Conjugate** — Requires Preparation x50
   Contents: DHEAS-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.8 mL/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 0.5 mL of HRP in 25 mL 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
A	0 µg/mL	2.0 mL
B	0.005 µg/mL	0.5 mL
C	0.02 µg/mL	0.5 mL
D	0.1 µg/mL	0.5 mL
E	0.5 µg/mL	0.5 mL
F	2.5 µg/mL	0.5 mL
G	10 µg/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.

4. **Controls** — Ready to Use
   - Contents: Two vials containing DHEAS in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of DHEAS. Refer to vial labels for the acceptable range.
   - Volume: 0.5 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vials 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.

5. **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 the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one 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 bottle containing a protein-based buffer with a non-mercury preservative.
   - Volume: 30 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

7. **TMB Substrate** — Ready To Use
   - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   - Volume: 16 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

8. **Stopping Solution** — Ready To Use
   - Contents: One bottle containing 1M sulfuric acid.
   - Volume: 6 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: None

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. **Prepare** working solutions of the DHEAS-HRP conjugate and wash buffer.

2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette **25 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette **200 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. **Incubate** on a plate shaker (~200 rpm) for 45 minutes at room temperature.
6. **Wash** the wells 3 times each time with 300 µL/well of diluted wash buffer per well. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of a washer is strongly recommended).
7. Pipette **150 µL** of the TMB substrate into each well at timed intervals.
8. **Incubate** on a plate shaker for 15-20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 7.
10. **Read** the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

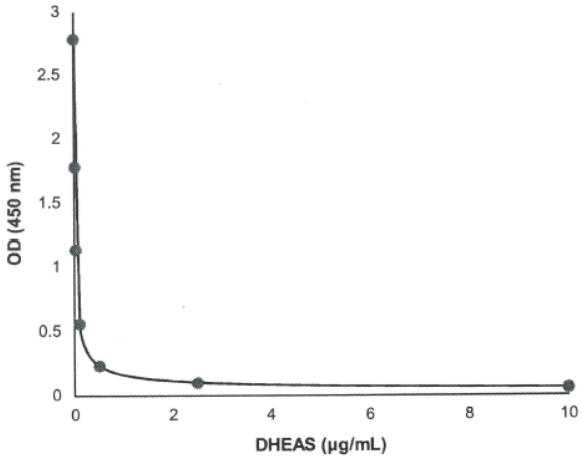
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density 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

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450nm)	% Binding	Value (µg/mL)
A	2.781	100%	0
B	1.776	64%	0.005
C	1.132	41%	0.02
D	0.558	20%	0.1
E	0.230	8%	0.5
F	0.100	4%	2.5
G	0.056	2%	10
Unknown	0.156	-	1.04

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Direct DHEAS ELISA kit is **0.005 µg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

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

Steroid	% Cross Reactivity
DHEAS	100
Androsterone	16.0
Androstenedione	1.7
Testosterone	0.9
Progesterone	0.6
DHT	0.6
Cortisol	0.5

The following steroids were tested but cross-reacted at less than 0.001%: 17β-Estradiol, Estrone, Estrone-Sulfate and Pregnenolone.

INTRA-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	0.24	0.02	7.5
2	2.02	0.18	8.9
3	9.54	0.11	11.5

INTER-ASSAY PRECISION

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

Sample	Mean	SD	CV %
1	0.13	0.02	15.3
2	1.11	0.09	8.1
3	6.38	0.27	4.2

RECOVERY

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

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	0.67	-	-
+ 0.1	0.84	0.77	109.1
+ 1.0	1.97	1.67	118.0
+ 5.0	5.80	5.67	102.3
2 Unspiked	1.21	-	-
+ 0.1	1.41	1.31	107.6
+ 1.0	2.01	2.21	91.0
+ 5.0	4.95	6.21	79.7
3 Unspiked	1.72	-	-
+ 0.1	1.93	1.82	106.0
+ 1.0	2.65	2.72	97.4
+ 5.0	5.45	6.72	81.1

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	2.88	-	-
1:2	1.74	1.44	120.8
1:4	0.88	0.72	122.2
1:8	0.43	0.36	119.4
2	6.32	-	-
1:2	3.17	3.16	100.3
1:4	1.63	1.58	103.2
1:8	0.78	0.79	98.7
3	7.12	-	-
1:2	3.09	3.56	86.8
1:4	1.54	1.78	86.5
1:8	0.80	0.89	89.9

EXPECTED NORMAL VALUES

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

Group	Range (µg/mL)
Males	0.39-4.63
Females	0.46-2.75
Postmenopausal Females	0.48-2.08

REFERENCES

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Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.