Package Insert

Dehydroepiandrosterone (DHEA) ELISA Assay Kit

Catalog Number:
DHA31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 9.2 (12 DEC 23)

EAGLE BIOSCIENCES, INC.
20A NORTHWEST BLVD., SUITE 112, NASHUA, NH 03063
PHONE: 617-419-2019 FAX: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

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

INTRODUCTION

Dehyroepiandrosterone (DHEA) is a C19 steroid produced in the adrenal cortex and to a lesser extent in the gonads. DHEA serves as precursor in testosterone and estrogen synthesis. Due to the presence of a 17-oxo-group, DHEA has relatively weak androgenic activity, which has been estimated at ~10% that of testosterone. However, in neonates, peripubertal children and in adult women, circulating DHEA levels may be several fold higher than testosterone concentrations, and rapid peripheral tissue conversion to more potent androgen (androstenedione and testosterone) and estrogen may occur. Moreover, DHEA has relatively low affinity for sex-hormone binding globulin. These factors may enhance the physiologic biopotency of DHEA. The physiologic role of DHEA has not been conclusively defined. A variety of in vivo and in vitro effects have been demonstrated, including antitumoral effects by provoking prevention and/ or regression in spontaneous or chemically induced skin and colon cancers in rodents. A few reports have suggested low production of DHEA(S) in women at risk of or having breast cancer. Therapeutic activity of DHEA has been reported for animals with diabetes of genetic origin, obesity and cardiovascular disease. There are also effects of DHEA in immune function, lipid metabolism, cholesterol, the nervous system, aging and in protection against virus development. Serum DHEA levels are relatively high in the fetus and neonates, low during childhood, and increase during puberty until the third decade of life. No consistent change in serum DHEA levels occur during the menstrual cycle or pregnancy. DHEA has a rapid metabolic clearance rate as compared to its sulfated conjugate, DHEA-S. Because of this, serum DHEA levels are 100–1000 fold lower than DHEA-S levels. In addition serum DHEA levels show significant diurnal variation which is dependent on adrenocorticotropic hormone (ACTH). Measurement of serum DHEA is a useful marker of adrenal androgen synthesis. Abnormally low levels may occur in hypoadrenalism, and elevated levels may occur in several conditions including virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3b-hydroxysteroid dehydrogenase deficiencies and in some case of female hirsutism.

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 TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of the DHEA present. The enzymatic reaction is terminated by addition of the stopping solution, converting the blue color to a yellow color. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of DHEA in the sample. A set of standards is used to plot a standard curve from which the amount of DHEA in patient samples and controls can be directly read.

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 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. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, 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.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of DHEA in human serum. The kit is not calibrated for the determination of DHEA 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 the DHEA sample diluent 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 0.2 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°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 single-channel pipettes to dispense 25 µL
2. Precision multi-channel pipettes to dispense 50, 100, and 150 µL
3. Precision pipette to dispense 350 µL (if washing manually)
4. Automatic microplate washer (recommended)
5. Disposable pipette tips
6. Distilled or deionized water
7. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)
8. DHEA Sample Diluent – If a sample concentration reads more than 40 ng/mL, then dilute the sample with the DHEA sample diluent at a dilution of no more than 1:4.

REAGENTS PROVIDED

1. **Rabbit Anti-DHEA 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. **DHEA-Horseradish Peroxidase (HRP) Conjugate** — Ready to Use
   - Contents: DHEA-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 14 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

3. **Dehydroepiandrosterone Calibrators** — Ready To Use

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

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

Calibrator	Concentration	Volume/Vial
A	0 ng/mL	1.0 mL
B	0.2 ng/mL	1.0 mL
C	1 ng/mL	1.0 mL
D	5 ng/mL	1.0 mL
E	15 ng/mL	1.0 mL
F	40 ng/mL	1.0 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 DHEA in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of DHEA. Refer to vial labels for the acceptable range.
   Volume: 1.0 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 distilled or deionized water.

6. **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.

7. **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 solution of the 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 100 µL of the DHEA-HRP conjugate into each well. (We recommend using a multichannel pipette.)
5. Gently tap the microplate frame for 10 minutes to mix the contents of the wells and incubate the microplate at room temperature (no shaking) for 90 minutes.
6. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a 3-cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: For manual washing, perform a 3-cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
7. Pipette 150 µL of the TMB substrate into each well (the use of a multi-channel pipette is recommended).
8. Gently tap the microplate frame for 10 seconds to mix the contents of the wells. Incubate the microplate for 10-15 minutes at room temperature.
9. Pipette 50 µL of stopping solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed 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 40 ng/mL then dilute it with the DHEA sample diluent at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

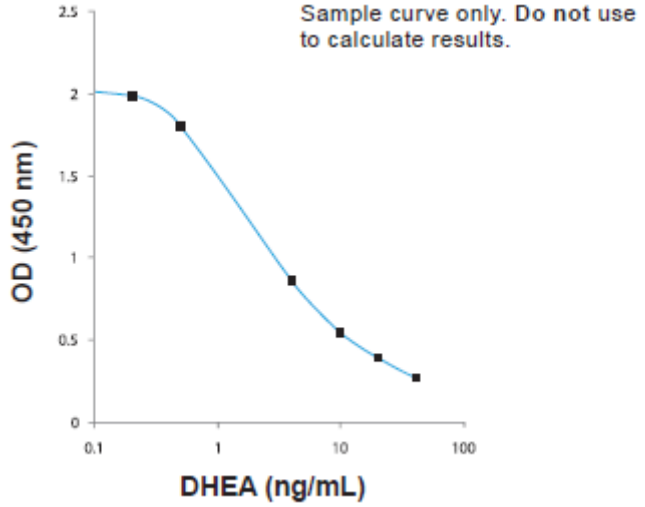
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	Value (ng/mL)
A	2.555	0
B	2.033	0.2
C	1.411	1
D	0.663	5
E	0.345	15
F	0.146	40
Unknown	1.025	2.14

TYPICAL CALIBRATOR CURVE

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



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB). Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	DHEA (ng/mL)
LoB	0.048
LoD	0.092

LoQ	0.13

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the DHEA ELISA kit with DHEA cross-reacting at 100%. ND=Not Detectable.

Steroid	% Cross Reactivity
DHEA	100
11-Deoxycortisol	0.17
17-Hydroxypregnenolone	2.09
17a-Hydroxyprogesterone	0.19
Aldosterone	0.11
Androstenedione	0.40
Androsterone	0.14
Cholesterol	ND
Corticosterone	0.12
Cortisol	0.07
DHEAS	<0.02
DHT	0.37
Epiandrosterone	2.49
Estradiol	0.49
Estrone	0.22
Pregnenolone	9.48
Progesterone	0.23
Testosterone	0.31

INFERENCES

An interference study was performed according to the CLSI EP07 guideline. No significant interference was observed for concentrations of up to 5 g/L haemoglobin, 40 mg/dL unconjugated bilirubin, 30 mg/dL conjugated bilirubin, 15 mg/mL triglycerides, 2.4 µL/mL HAMAS, 2.4 µL/mL Bioten and 1688 IU/mL Rheumatoid Factor.

PRECISION

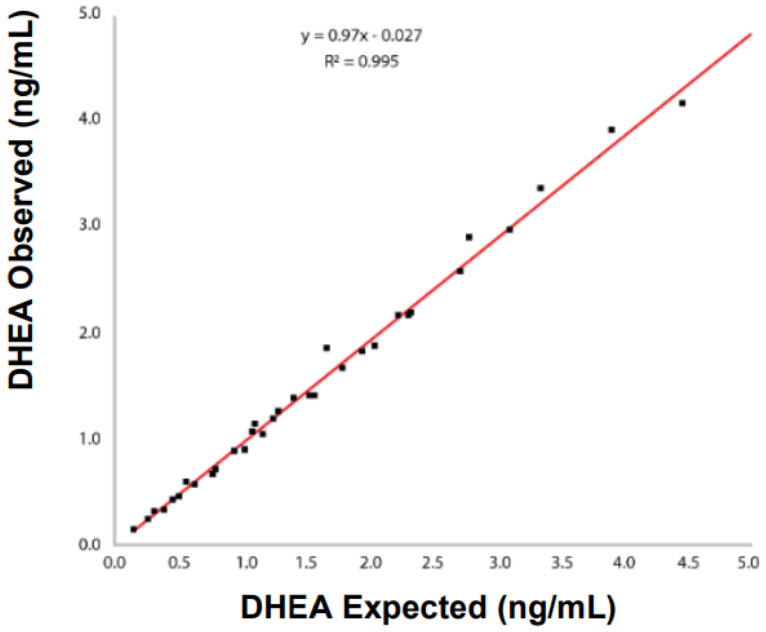
The precision study was performed according to the CLSI EP5-A3 guideline. The experimental protocol used a nested components-of-variance design with 8 serum samples. 10 testing days, two lots and two operators per day. Each operator ran two tests with two lots per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) using human serum samples. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	Within Run SD	Within Run CV %	Between Run SD	Between Run CV%	Total SD	Total CV%
1	1.20	0.05	3.8	0.12	10.2	0.13	10.9
2	3.50	0.09	2.7	0.29	8.3	0.31	8.7
3	8.88	0.25	2.8	0.54	6.1	0.64	7.2
4	3.26	0.10	3.0	0.27	8.4	0.29	9.0

5	2.81	0.10	3.5	0.25	8.7	0.26	9.4
6	1.38	0.04	3.2	0.14	10.1	0.16	11.5
7	13.28	0.36	2.7	0.95	7.1	1.08	8.1
8	20.20	0.51	2.5	1.65	8.2	1.73	8.6

LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following the CLSI EP06-A guideline. The samples were diluted in the DHEA Sample Diluent at several equidistant concentration levels and up to ten percent (1:10), tested in quadruplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using the DHEA sample diluent as the diluent.



RECOVERY

Low-value samples and high-value samples were mixed at different rations and measured with the DHEA ELISA kit. The recovery results from twelve samples were between 90 and 110%.

COMPARATIVE STUDIES

The DBC DHEA ELISA kit (y) was compared against a Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method (x). The comparison of 98 serum samples yielded the following Passing-Bablok regression:
Y = 0.65x + 0.61, r = 0.923

REFERENCE RANGES

Reference ranges were established using serum samples from 264 female donors between 18-63 years old and 130 male donors between 18-65 years old. The reference ranges were determined using a non-parametric method and are summarized in the table below.
Each laboratory shall establish their own reference ranges.

Adult	Age (years)	N	Median (ng/mL)	Mean (ng/mL)	95% Reference Range (ng/mL)
Males	18-65	130	2.80	3.04	1.33-6.48
Females	18-63	264	2.35	2.61	1.00-5.86

Children	Age (years)	N	Total Range* (ng/mL)
Males	1-9	28	0.20-1.5
Males	10-14	23	0.58-3.7
Males	15-18	14	1.50-3.6
Females	2-9	27	0.36-3.6
Females	10-14	21	0.47-5.5
Females	15-18	19	0.41-5.7

*****Since the number of pediatric samples is insufficient to establish a 95% reference range, the total range is provided which shows the lowest to the highest value obtained in each age group.

REFERENCES

1. Chaslow FI, Blethen SL, Bradlow HL. Dehydroepiandrosterone Sulfate (DHEAS) and DHEAS-like Compounds in Fibrocystic Disease of the Breast. *Steroids.* 1988; 52(3):205-15
2. Chasalow FL, et al. Serum Levels of Dehydroepiandrosterone Sulfate as Determined by Commercial Kits and Reagents. *Steroids.* 1989; 54(4):373-81.
3. dePeretti E, Forest MG. Pattern of Plasma Dehydroepiandrosterone Sulfate Levels in Humans from Birth to Adulthood: Evidence for Testicular Production. *J Clin Endocrinol Metab.* 1978;47(3):572-77
4. Holtzclaw WD, Gordon GB. Measurement of Serum Levels of Dehydroepiandrosterone Sulfate: A Comparison of Radioimmunoassay and Enzymatic Analysis. *Steroids.* 1989; 54(4): 355-71
5. Koritnik DR, et al. A Radioimmunoassay for Dehydroepiandrosterone Sulfate in the Circulation of Rhesus Monkeys. *Sterioids.* 1983; 42(6)653-67
6. Orentreich N, et al. Age Changes and Sex Differences in Serum Dehydroepiandrosterone Sulfate Concentrations Throughout Adultgood. *J Clin Endocrol Metab.* 1984; 59(3) 551-55.
7. Smith MR, et al. A Radioimmunoassay for the Estimation of Serum Dehydroepiandrosterone Sulphate in Normal and Pathological Sera. *Clin Chim Acta.* 1975;65(1) 5-13.
8. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. *Gynecol Obstet Invest.* 1995; 40(2):139-40

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.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damage of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

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.