CE

DiaMetra
care for quality

DCM124-1
Ed. 03/2012

DHEA ELISA

for routine analysis

Immunoenzymatic determination of DHEA in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO124

INTENDED USE

Eagle Biosciences DHEA ELISA Assay Kit is a competitive immunoenzymatic colorimetric method for quantitative determination of Dehydroepiandrosterone (DHEA) concentration in human serum or plasma. DHEA ELISA Assay Kit is intended for research use only and is not intended for diagnostic procedures.

1. CLINICAL SIGNIFICANCE

Dehydroepiandrosterone (DHEA) is a C19 steroid secreted by the adrenal cortex and in smaller quantities by the gonads. DHEA is a precursor in testosterone and various estrogens biosynthesis.
The physiologic role of DHEA is not-well defined, because of a lot of ascertained in vivo and in vitro effects, for example the prevention and regression of colon tumor, spontaneous and inducted, in rodents.
Some studies showed a decrease in the DHEA production in women with risk for the breast cancer. In animal models DHEA showed a role in the therapeutic action against diabetes, obesity and cardiovascular pathologies, and unclear roles in immunology, in lipid metabolism (also cholesterol) and in the nervous system.
Seric levels of DHEA are relatively high in fetal and neonatal age, decrease during childhood and increase again during puberty until 30 years. No DHEA variations have been observed during pregnancy or menstrual cycle. Seric levels of DHEA are 100-1000 times lower than the levels of DHEA sulphate (DHEA-S). The measurement of seric levels of DHEA is a marker for adrenal androgen synthesis. Abnormal low levels of DHEA can be observed in the hypoadrenalism, while elevated levels can be observed in various pathologies such as the surrenalic adenomas, 21-hydroxylase and 3b-hydroxysteroid dehydrogenase deficiency and in some cases of female hirsutism.

2. PRINCIPLE OF THE METHOD

In the DHEA ELISA Assay Kit, the essential reagents required for this immunoenzymatic assay are a biotinylated anti DHEA antibody, the DHEA antigen conjugated with the enzyme HRP (horseradish peroxidase), the DHEA antigen present in the sample, and a microplate coated with Streptavidin (highly specific for Biotin). The quantity of native antigen is unknown, while the antibody and the antigen linked to HRP are in excess.
In the first part of the DHEA ELISA Assay Kit these three components are mixed together; a competitive reaction for the anti DHEA antibody between the native antigen and the antigen linked to HRP develops.

The interaction is illustrated by the following equation:

$$EAg + Ag + Ab_{BT} \underset{k\text{-}a}{\overset{ka}{\Leftrightarrow}} Ag\text{-}Ab_{BT} + EAg\text{-}Ab_{BT}$$

EAg = antigen linked to HRP enzyme
Ag = native antigen (unknown amount in the sample)
Ab_{BT} = biotinylated anti DHEA antibody
$Ag\text{-}Ab_{BT}$ = native antigen-antibody complex
$EAg\text{-}Ab_{BT}$ = native antigen linked to HRP - antibody complex
ka = rate constant of association
k-a = rate constant of dissociation

Simultaneously to their formation, the complexes are fixed to the microplate wells through the interaction between the Streptavidin and the biotinylated antibody.
The DHEA ELISA Assay Kit reagents in excess that have not reacted are eliminated in the washing steps.
In the last part of the assay, the enzyme HRP linked in the wells reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H_2SO_4) is added. The color intensity is inversely proportional to the native antigen DHEA in the sample. DHEA concentration in the sample is finally calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. DHEA Calibrators (6 vials, 1 mL each)

CAL0	**REF DCE002/12406-0**
CAL1	**REF DCE002/12407-0**
CAL2	**REF DCE002/12408-0**
CAL3	**REF DCE002/12409-0**
CAL4	**REF DCE002/12410-0**
CAL5	**REF DCE002/12411-0**

2. Enzyme Reagent (2 vials, 3 mL each)
DHEA conjugated with horseradish peroxydase (HRP)
REF DCE002/12402-0

3. Biotin Reagent (2 vials, 3 mL each)
Antibody anti DHEA biotinylated
REF DCE019/12419-0

4. Coated Microplate (1 breakable microplate)
Microplate coated with Streptavidin
REF DCE002/12403-0

5. TMB Substrate (1 vial, 12 mL)
3,3',5,5'-tetramethylbenzidine, hydrogen peroxide
(avoid any skin contact) **REF DCE004/12404-0**

6. Stop Solution (1 vial, 8 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005/12405-0

7. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L
REF DCE006/12406-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Notes

Store all reagents between 2- 8°C in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS

- This DHEA ELISA Assay Kit is intended for research use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators should be handled in the same manner as potentially infectious material.
- Some reagents of the DHEA ELISA Assay Kit contain small amounts of Proclin 300^{R} as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the DHEA ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all DHEA ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange DHEA ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Calibrators (C_0…C_5)

The Calibrators are ready for use and have the following concentration of DHEA:

	C_0	C_1	C_2	C_3	C_4	C_5
ng/mL	0	0.5	2.0	5.0	10.0	30.0

Once opened, the Calibrators are stable for 6 months at 2-8°C.

6.2. Preparation of the Sample

The determination of Dehydroepiandrosterone can be performed in human plasma as well as in serum samples.
Store the sample at -20°C if the determination is not performed on the same day of the sample connection. Avoid repetitive freezing and thawing of samples.

6.3. Preparation of Wash Solution

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

6.4. PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample	Blank
Calibrator C_0-C_5	25 µL		
Sample		25 µL	
DHEA Enzyme Reagent	50 µL	50 µL	
Shake gently the microplate for 20-30 seconds to mix.			
DHEA Biotin Reagent	50 µL	50 µL	
Shake gently the microplate for 20-30 seconds to mix. Cover the plate. Incubate 1 h at room temperature (22-28°C). Remove the contents from each well; wash the wells 3 times with 350 µL of diluted Wash Solution.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate 20 minutes at room temperature (22÷28°C), in the dark.			
Stop Solution	50 µL	50 µL	50 µL
Shake gently the micoplate. Read the absorbance (E) at 450 nm against blank.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of DHEA for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbances (Em) for each point of the calibration curve (C_0-C_5) and of each sample.

8.2. Calibration Curve

Plot the values of absorbance (Em) of the calibrators (C_0-C_5) against concentration.
Draw the best-fit curve through the plotted points(es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

9. REFERECE VALUES

The serum or plasma Dehydroepiandrosterone reference values are:

	Female	Male
Range (ng/mL)	1.3 – 9.8	1.8 – 12.5

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra-Assay Variation

Within run variation was determined by replicate the measurement of three different control sera in one assay. The within assay variability is ≤ 9,8%.

10.1.2. Inter-Assay Variation

Between run variation was determined by replicate the measurement of three different control sera in different lots. The between assay variability is ≤ 10,7%.

10.2. Sensitivity

The lowest detectable concentration of DHEA that can be distinguished from the Calibrator 0 is 0.10 ng/mL at the 95% confidence limit.

10.3. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Substance	%
DHEA	100
DHEA-S	0,004
Androstenedione	0,056
Corticosterone	0,004
Cortisol	0,001
Pregnenolone	0,070
Testosterone	0,002
Dihydrotestosterone	0,007
Estriol	< 0,001
Estradiol	< 0,001
Estrone	< 0,001

11. WASTE MENAGEMANT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1. Dorfman RI, Shipley, RA, *Adrogens*, John Wiley and Sons, New York, 1956, pp. 116-128.
2. Pang S, Riddick L, Hirsutism, IN Lifshitz (ed), *Pediatric Endocrinology, A Clinical Guide, second edition*, Marcel Dekker Inc., New York 1990, pp 259-291
3. De Peretti E, Forest MG, Pattern of plasma dehydroepiandrosterone sulfate levels ih humans from birth to adulthood: evidence for testicular production, *J Clin Endocrinol Metab*, 47, 572-577 (1978)
4. Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Di Martino-Nardi J, Reiter E, Normative data for adrenal steroidogenesis in a healthy pediatric population: age and sex-related changes after adrenocorticotropin stimulation, *J Clin Endocrinol Metab*, 73, 674-686 (1991)
5. Zurnoff B, Roenfeld RS, Stain GW, Levin J, Fukushima DK, Sex differences in twenty-four hour mean plasma concentration of dehydroepian-drosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) and the DHEA to DHEAS ratio in normal adults, *J Clin Endocrinol Metab* 51, 330-333 (1980)

Ed. 03/2012 **DCM124-1**

DiaMetra S.r.l. Headquater: Via Garibaldi, 18 – 20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039–02–2133354.
Manufactory: Via Pozzuolo 14, 06038 SPELLO (PG) Italy
Tel. 0039-0742–24851
Fax 0039–0742–316197
E-mail: info@diametra.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

EAGLE BIOSCIENCES

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation