AccuDiag™ DHEA-S ELISA Kit

REF 2055-17

IVD See External Label 2°C 8°C Σ 96 Tests

Dehydroepiandrosterone Sulphate DHEA-S ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0-10 µg/mL
Sample	30 µL serum/plasma
Incubation Time	75 minutes
Shelf Life	12 Months from the manufacturing date
Specificity	See Table
Sensitivity	0.04 µg/ml

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. DHEA-S ELISA kit is competitive immunoenzymetic colorimetric method for quantitative determination of DHEA-S concentration in human serum or plasma. DHEA-S is intended for laboratory use only.

SUMMARY AND EXPLANATION

Dehydroepiandrosterone sulfate (DHEA-S), is a natural steroid hormone found atop of the kidneys in the human body. DHEA-S derived from enzymatic conversion of DHEA in adrenal and extradrenal tissues. DHEA-S is also produced in the gonads, adipose tissue and the brain. It is the most abundant hormone in the human body and it is precursor of all sex steroids.

As most DHEA-S is produced by the zona reticularis of the adrenal, it is argued that there is a role in the immune and stress response. DHEA-S may have more biologic roles. Its production in the brain suggests that is also has a role as a neurosteroid.

Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels may occur in have been reported in hypoadrenalism, while elevated levels occur in several conditions, e.g. virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies and in some cases of female hirsutism. Women with polycystic ovary syndrome tend to have normal or mildly elevated levels of DHEAS. As very little DHEA-S is produced by the gonads, measurement of DHEA-S levels may aid in the localization of androgen source in virilizing conditions. DHEA-S levels show no diurnal variation.

ASSAY PRINCIPLE

The DHEA-S (antigen) in the sample competes with the antigenic DHEA-S conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti DHEA-S coated on the microplate (solid phase). After the incubation, the bound/free separation is performed by a simple solid-phase washing.

Then, the enzyme HRP in the bound-fraction 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 DHEA-S concentration of in the sample.
DHEA-S concentration in the sample is calculated through a standard curve.

REAGENTS

Materials provided with the test kit

Reactive Reagents

1. DHEA-S Standards 6x (1 vial = 1 mL ready to use)
   STD0
   STD1
   STD2
   STD3
   STD4
   STD5
2. Serum diluent (1 vial) 100 mL
   HEPES 187 mM pH 7.5; BSA 0.5 g/L
3. Conjugate (1 bottle) 12 ml
   DHEA-S conjugate with horseradish peroxidase (HRP)
4. Coated Microplate (1 microplate breakable)
   Antibody anti DHEA-S adsorbed on microplate
5. TMB-substrate (1 vial) 15 ml
   H_2O_2-TMB 0.26 g/L (avoid any skin contact)
6. Stop solution (1 vial) 15 ml
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
7. DHEA-S Control (2 vials) 1 mL each
   Control A
   Control B
   Concentration of the Control is indicated on the Certificate of Analysis
8. 10X Conc. Wash Solution (1 vial, 50 ml)
   Phosphate buffer 0.2M pH 7.4

Materials required but not provided

1. Distilled water
2. Automatic dispenser
3. Microplates reader (450 nm,620-630 nm)

Notes

Store all reagents between 2-8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit. Do not remove the adhesive sheets on the unused strips.

REAGENT PREPARATION

1. **Preparation of Controls and Standard (S_0,S_1,S_2,S_3,S_4, S_5)**
   The standards have the following concentration of DHEA-S:

	S_0	S_1	S_2	S_3	S_4	S_5
µg/ml	0	0.1	0.4	1.0	4.0	10.0

   The Standards are ready to use and stable until the expiry date printed on the label. Once opened, the standards are stable for 6 months at 2-8°C.The controls are ready to use.
2. **Preparation of the Sample**
   The determination of Dehydroepiandrosterone Sulphate can be performed in human plasma as well as in serum of patients.
   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.

   Immediately before use, dilute each sample 1:50 with diluted Serum Diluent (i.e. add to 980 µL of diluted Serum Diluent 20 µL of sample). Mix well.
3. **Preparation of Wash Solution**
   Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.
   In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

ASSAY PROCEDURE

1. **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
2. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
3. 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 standard curve (S_0-S_5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample	Blank
Diluted Sample/Control		**30 µL**	
Standards S_0-S_5	**30 µL**		
Conjugate	**100 µL**	**100 µL**	
Incubate at 37°C for *1 hour*. Remove the contents from each well. Wash the wells 2 times with 300 µL of distilled water. **Important note:** during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer:** if you use automated equipment, wash the wells at least 5 times.			
TMB substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22-28°C) for 15 minutes in the dark.			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

RESULTS

1. **Mean Absorbance**
   Calculate the mean of the absorbances (Em) for each point of the standard curve (S_0-S_5) and of each sample.
2. **Standard Curve**
   Plot the values of absorbance (Em) of the Standards (So-S5) against concentration.
   Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).
3. **Calculation of Results**
   Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in µg/mL.

REFERENCE VALUE

The serum or plasma Dehydroepiandrosterone Sulphate reference values are:

	WOMAN µg/mL	MAN µg/mL
Newborns	0.9 - 1.8	0.9 - 1.8
Before puberty	0.25 - 1.0	0.25 - 1.0
Adults	0.9 - 3.6	0.9 - 3.6
After menopause	< 0.25 - 1.0	
Pregnancy	0.25 - 1.8	

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.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of DHEA-S 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.

PERFORMANCE CHARACTERISTICS

Precision

1. **Intra-Assay Variation**
   Within run variation was determined by replicate (16X) the measurements of three different serum samples on one assay. The within assay variability is <7.9%.
2. **Inter-Assay Variation**
   Between run variation was determined by replicate (20x) the measurements of three different control sera in different lots. The between assay variability is <10.4%.
3. **Accuracy**
   The recovery of 0.6 – 1.25 – 2.5 and 5 µg/mL of DHEA-S added to sample gave an average value (±SD) of 102.87% ± 8.63% with reference to the original concentrations. The dilution test performed on three sera diluted 2 – 4 – 8 times gave an average value (±SD) of 100.15% ± 9.02%.
4. **Sensitivity**
   The lowest detectable concentration of DHEA-S that can be distinguished from the standard 0 is 0.04 µg/ml at the 95 % confidence limit.
5. **Specificity**
   The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

DHEA-S	100%
DHEA	100%
Androsterone	16%
Androstenedione	59%
DHT	1.0%
Pregnenolone	0.18%
Testosterone	0.63%
Progesterone	0.27%
17 OH Progesterone	0.15%
Estrone	0.3%
Cortisol	<0.01%
Cholesterol	<0.001%
11-deoxycortisol	<0.08%
17b Estradiol	<0.001%
Corticosterone	<0.01%
Cortisone	<0.013%
Estriol	<0.001%
Estradiol Sulphate	<0.001%
Aldosterone	<0.001%
Estradiol-3-Sulphate-17 glucuronide	<0.001%

6. **Specificity: interfering substances**
   Interference by Bilirubin, Hemoglobin and Triglyceride has been investigated on DAI DHEA-S ELISA kit:

Substance	Assayed Conc.	Interference
Bilirubin	0.2 mg/ml	No
Hemoglobin	2 mg/ml	No
Triglycerides	6 mg/ml	No

No Interference has been obscured with the substances under investigation; following good laboratory practices, it is anyway advised to avoid to use highly lipemic or hemolyzed samples.

7. **Specificity: Plasma and SST tube**
   Interference in plasma and SST (serum separation tube) samples has been evaluated. Serum obtained from the same patient has been used as reference.

Sample	Interference
SST (serum separation tube)	No
EDTA plasma	No
Lithium heparin plasma	No
Sodium heparin plasma	No

No interference has been observed.

8. **Correlation with RIA**
   The DAI DHEA-S ELISA was compared to another commercially available DHEA-S assay. Serum samples of 42 serum samples have been analyzed according in both test systems.
   The linear regression curve was calculated
   $Y = 0.93x + 0.28$
   $r^2 = 0.961$

WARNINGS

1. This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. Some reagents contain small amounts of Proclin 300® as preservatives. Avoid the contact with skin or mucosa.
5. 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.
6. 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.
7. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
8. This method allows the determination of Dehydroepiandrosterone Sulphate from 0.1 µg/mL to 10 µg/mL.
9. The clinical significance of the determination Dehydroepiandrosterone Sulphate can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PRECAUTIONS

1. 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.
2. All reagents 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.
3. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
4. Do not interchange 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.
5. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
6. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
7. 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

8. 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.
9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

REFERENCES

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2. Granoff A.B. et al Obstet. Gynecol., 53 (1), 111 (1979)
3. Hopper B.R.et al J. Clinic. Endocrin. Metab. 40 (3), 458 (1975)
4. Winter J.S.D, et al Clinic. Obstetic and Gynecol., 21 (1), 67 (1978)

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Date Adopted	**2022-09**
REF 2055-17	**AccuDiag™ - DHEA-S ELISA**
Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu**
Revision Date: 2018-09	