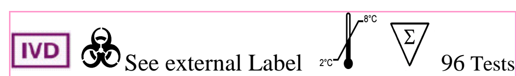
CE

AccuDiag™ DHEA-S ELISA Kit

Cat# 2055-17



Test	Dehydroepiandrosterone Sulphate DHEA-S ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0-03 / 10 ug/mL
Sample	30 µL serum/plasma
Total Time	~ 75 min.
Shelf Life	12 -14 Months from the manufacturing date
Specificity	95 %
Sensitivity	0.03 µg/ml

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.

TEST PRINCIPLE

Dehydroepiandrosterone Sulphate (antigen) in the sample competes with horseradish peroxidase (HRP) for binding onto the limited number of anti-dehydroepiandrosterone sulphate (antibody) sites on the microplates (solid phase). After 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 is added. The color intensity is inversely proportional to the dehydroepiandrosterone sulphate concentration in the sample. DHEA-S concentration in the sample is calculated through a calibration curve.

Reagent Preparation

1. **Preparation of Standard (S_0,S_1,S_2,S_3,S_4, S_5)**
The standard has the following concentration of DHEA-S:

	S_0	S_1	S_2	S_3	S_4	S_5
ng/ml	0	2	8	20	80	200

Stability: until the expiration date printed on the kit.

The standard concentration are 50 times lower than the values reported in the reference range because in this method the samples are diluted 1/ 50 while the standards are not diluted. The concentrations to be entered in the instruments for calculations are:

	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**

Once open the standards are stable for six month at +4°C.

2. **Preparation of Serum diluent**
Dilute contents of 5X Serum diluent Conc. to 100ml with distilled or deionized water in a suitable storage container. Store at room 2 – 8°C until expiration date printed on label.

3. **Preparation of the Sample**
The determination of Dehydroepiandrosterone Sulphate can be performed in plasma as well as in serum of patients who have observed fast. Store the sample at -20°C if the determination is not performed on the same day of the sample connection.
Immediately before use, dilute each sample 1:50 with diluted serum Diluent (i.e. add to 980 uL of diluted Serum Diluent 20 uL of sample). Mix well. The Control is ready for use.

MATERIALS AND COMPONENTS

Materials provided with the test kits

Reactive Reagents

1. DHEA-S Standards 6x (1 vial = 1 mL)

STD_0 **REF DAS0/2055-17**
STD_1 **REF DAS1/2055-17**
STD_2 **REF DAS2/2055-17**
STD_3 **REF DAS3/2055-17**
STD_4 **REF DAS4/2055-17**
STD_5 **REF DAS5/2055-17**

1. Serum diluent Conc. 5X (1 bottle) 20 mL
HEPES 187 mM pH 7.5; BSA 0.5 g/L **REF DA-D/2055-17**

2. Conjugate (1 bottle) 12 ml
DHEA-S-HRP conjugate **REF DA-C/2055-17**

3. Coated Microplate (1 microplate breakable)
Anti-Dehydroepiandrosterone Sulphate IgG adsorbed on microplate
REF DA-P/2055-17

4. TMB-substrate (1 bottle) 15 ml
H_2O_2-TMB 0.26 g/L
(avoid any skin contact) REF **DA-T/2055-17**

5. Stop solution (1 bottle) 15 ml
Sulphuric acid 0.15 mol/L
(avoid any skin contact) REF **DA-S/2055-17**

7. DHEA-S Control (1 bottle) 1 mL
Concentration of the Control is Lot-Specific and is indicated on the Certificate of Analysis **REF DA-Cont/2055-17**

Materials required but not provided

1. Distilled water.
2. Automatic dispenser.
3. Microplates reader

Notes

Store all reagents between 2÷8C°in the dark.
Open the bag of reagent 3 (Antibody) only when it is at room temperature and close immediately after use. Do not remove the adhesive sheets on the unused strips.

ASSAY PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_5), two for each sample, one for Blank.

Reagent	Standard	Sample	Blank
Standard S_0-S_5	**30 µL**		
Diluted sample/Control		**30 µL**	
Conjugate	**100 µL**	**100 µL**	
Incubate at 37°C for *1 hour*. Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely.			
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**
Read the absorbance (E) at 450 nm against Blank.			

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 of the standards against concentration.
Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).
The concentrations to be entered in the instruments for calculations are:

	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

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	

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 determination of four different control sera in one assay. The within assay variability is <5.7%.

2. **Inter-Assay Variation**
Between run variation was determined by replicate (16x) the measurements of three different control sera in 2 different lots. The between assay variability is <9.6%.

3. **Accuracy**
The recovery of 5 – 2.5 – 1.25 – 0.6 µg/mL of DHEA-S added to sample gave an average value (±SD) of 96.71% ± 12.02% with reference to the original concentrations. The dilution test performed on three sera diluted 2 – 4 – 8 times gave an average value (+/-SD) of 98.19%+/- 5.70%.

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

DHEA-S	90 %
DHEA	100.0 %
Androsterone-S-Na	48 %
Androstenedione	20 %
Etiocolanone-S-Na	0.2 %
5-Androstendione	0.01 %
Testosterone	0.01 %
Progesterone	0.01 %
17 OH Progesterone	0.01 %
Estrone	0.01 %
Cortisol	0.001 %
Colesterolo	0.001 %

5. **Sensitivity**
The lowest detectable concentration of Dhea-s that can be distinguished from the zero standard is 0.03 µg/ml at the 95 % confidence limit.

6. **Correlation with RIA**
The DHEA-s ELISA was compared to another commercially available DHEA-s assay. Serum samples of 29 females and 20 males were analyzed according in both test systems.
The linear regression curve was calculated
(DHEA-S)=0.94*(DHEA-S RIA) – 0.01

$r^2 = 0.906$

LIMITATIONS OF THE PROCEDURE

1. Assay Performance
Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. 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 one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

2. Interpretation
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

PRECAUTIONS

1. The reagent contains Proclin 300 as preservative.
2. All reagents should be 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. Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants
5. Maximum precision is required for reconstitution and dispensation of the reagents.
6. Do not use different lot reagent.
7. This method allows the determination of Dehydroepiandrosterone Sulphate from 0.1 µg/mL to
8. 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.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES

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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)