



DIAGNOSTIC AUTOMATION, INC.

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See external label



2°C-8°C



Σ=96 tests



Cat #2055Z

Direct Immunoenzymatic Determination of Dehydroepiandrosterone Sulphate in Serum or Plasma.

DHEA-S

Cat # 2055-Z

Test	Dehydroepiandrosterone Sulphate DHEA-S ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-200 ng /ml
Sample	20ul serum
Specificity	95%
Sensitivity	4.5 ng/ml
Total Time	~ 75 min
Shelf Life	12-14 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The Diagnostic Automation Inc. DHEA-S ELISA kit is competitive immunoenzymatic colorimetric method for quantitative determination of Dhea-S concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

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 extrarenal 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 it 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 3 β -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.

PRINCIPLE

Dehydroepiandrosterone Sulphate (antigen) in the sample competes with horseradish peroxidase dehydroepiandrosterone sulphate (enzyme-labelled antigen) 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.

The enzyme substrate (H₂O₂) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined.

Dehydroepiandrosterone Sulphate concentration in the sample is calculated based on a series by a set of standard.

The colour intensity is inversely proportional to the dehydroepiandrosterone sulphate concentration in the sample.

REAGENT, MATERIAL AND INSTRUMENTATION

1. Reagent and material supplied in the kit

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

STD ₀	REF	DAS0/2055Z
STD ₁	REF	DAS1/2055Z
STD ₂	REF	DAS2/2055Z
STD ₃	REF	DAS3/2055Z
STD ₄	REF	DAS4/2055Z
STD ₅	REF	DAS5/2055Z

2. Serum diluent Conc. 5X (1 bottle) 20 mL

HEPES 25 mM pH 7.4; BSA 0.5 gr/L

REF DA-D/2055Z

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

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

5. TMB-substrate (1 bottle) 15 ml
H₂O₂-TMB 0.25gr/L
(avoid any skin contact) **REF DA-T/2055Z**

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

2. Reagents necessary not supplied

Distilled water.

3. Auxiliary materials and instrumentation

Automatic dispenser.

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

PRECAUTION

- The reagent contain Proclin 300 as preservative.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lot reagent.
- This method allows the determination of Dehydroepiandrosterone Sulphate from 0.1 µg/mL to 10 µg/mL.
- The clinical significance of the determination Dehydroepiandrosterone Sulphate can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PROCEDURE

1. Preparation of Standard (S₀,S₁,S₂,S₃,S₄, S₅)

The standard has the following concentration of Dehydroepiandrosterone Sulphate:

	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:

	S₀	S₁	S₂	S₃	S₄	S₅
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 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 prepare a tube for each sample and pipette 20 µl of the Sample in 1.0 mL of Serum Diluent (diluted). Mix well.

PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S₀-S₅), two for each sample, one for Blank.

Reagent	Standard	Sample	Blank
Standard S ₀ -S ₅	30 µL		
Diluted sample		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	
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.			

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.

LIMITATION OF PROCEDURE

1. Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic 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.

RESULTS

1. Mean Absorbance

Calculate the mean of the absorbances (E_m) 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
$\mu\text{g/ml}$	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 $\mu\text{g/mL}$.

REFERECE 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	

PERFORMANCE AND CHARACTERISTICS

Precision

1. Intra-Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4.8%.

2. Inter-Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8.9%.

3. Accuracy

The recovery of 5 – 2.5 – 1.25 µg/mL of DHEA-S added to sample gave an average value (±SD) of 104.6% ± 2.2% with reference to the original concentrations.

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 0.045 µg/ml at the 95 % confidence limit.

6. Correlation with RIA

The Dia.metra Dhea-s ELISA was compared to another commercially available Dhea-s assay. Serum samples of 30 females and 19 males were analysed according in both test systems.

The linear regression curve was calculated

$$y = 0.94 x - 0.02$$

$$r = 0.9887 \text{ (} r^2 = 0.977 \text{)}$$

WASTE MENAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1. Abraham G.E., et al Obstet. Gynecol., 47 (4), 395 (1976)
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)

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