

REFERENCES

1. Tietz, N. W., Textbook of Clinical Chemistry, Saunders, 1968

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**DHEA-S ELISA**

Catalog No. DH291S (96 Tests)

Cat#: DH291S (96 Tests)
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INTENDED TO USE

The Calbiotech Inc. (CBI) DHEA-S ELISA kit is used for the quantitative measurement of DHEA-S in human serum and plasma.

SUMMARY AND EXPLANATION

DHEA-S is a more specific product of the adrenals and measurements of this steroid are widely intended in clinical practice. The clinical importance of plasma assays of DHEA-S is associated with the diagnosis of adrenal hyperplasia and differential diagnosis of hirsutism.

PRINCIPLE OF THE TEST

The CBI DHEA-S kit is based on the principle of competitive binding between DHEA-S in the test specimen and HRP conjugated DHEA-S for a constant amount of anti-DHEA-S antibody. In the first incubation, Goat-anti-Rabbit-IgG coated wells are incubated with 10µl of DHEA-S standards, patient samples, 50µl DHEA-S Enzyme (HRP) reagent and 50µl anti-DHEA-S Antibody reagent, at room temperature, for 60 minutes. During the incubation, HRP labeled DHEA-S competes with the endogenous DHEA-S in the standard and sample, for a fixed number of binding sites of the DHEA-S antibody, while simultaneously the Anti DHEA-S antibody binds to the immobilized secondary antibody. Thus, the amount of DHEA-S HRP conjugate immunologically bound to the well progressively decreases as the concentration of DHEA-S in the specimen increases. Unbound DHEA-S HRP conjugate is then removed and the wells washed. Next, TMB Reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is spectrophotometrically measured at 450nm. A standard curve is prepared relating color intensity to the concentration of DHEA-S.

MATERIALS PROVIDED	96 Tests
Microwells coated with Goat anti-Rabbit IgG	12x8x1
Standard set, 7 vials (ready to use)	0.25 ml
DHEA-S Enzyme Reagent, 1 bottle (ready to use)	6 ml
Anti- DHEA-S Antibody Reagent, 1 bottle (ready to use)	6 ml
TMB Substrate (ready to use)	12 ml
Stop Solution (ready to use)	12 ml
Wash solution, 1 bottle (20X)	25 ml

MATERIALS NOT PROVIDED

1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

1. **Prepare 1X Wash Buffer** by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use.

1. Secure the desired number of coated strips in the holder.
2. Dispense 10 µl of each standards, controls and sample with new disposable tips into appropriate wells.
3. Dispense 50 µl of DHEA-S Enzyme-Reagent into each well.
4. Dispense 50 µl of Anti-DHEA-S Antibody-Reagent into each well.
5. Thoroughly mix the plate for 10 seconds. It is important to have complete mixing in this step.
6. Incubate for 60 minutes at room temperature.
7. Briskly shake out the contents of the wells.
8. Rinse the wells 3 times with diluted Wash Solution (350 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add 100 µl of TMB Substrate into each well.
10. Incubate for 30 minutes at room temperature.
11. Stop the enzymatic reaction by adding 50 µl of Stop Solution into each well.
12. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check DHEA-S standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for DHEA-S standards (vertical axis) versus standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	Conc. µg/mL	OD 450 nm
Std 1	0	2.02
Std 2	0.1	1.34
Std 3	0.5	0.95
Std 4	1	0.70
Std 5	2.5	0.40
Std 6	5	0.24
Std 7	10	0.13

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.