

DHEA-S (human) ELISA Kit

(Catalog # K7428-100, 100 assays; Store at 2-8°C)

rev 08/18

I. Introduction:

DHEA-S (Dehydroepiandrosterone sulfate) is a metabolite of DHEA (Dehydroepiandrosterone). It is more specific product of the adrenals and measurements of this steroid is 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. BioVision's human DHEA-S kit is based on the principle of competitive binding between DHEA-S in the sample 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 DHEA-S Standards, samples, DHEA-S Enzyme (HRP) reagent and anti-DHEA-S Antibody reagent, at room temperature. 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 sample increases. Unbound DHEA-S HRP conjugate is removed and wells are washed. Next, TMB Reagent is added and incubated at room temperature, resulting in the development of blue color. The color development is stopped with addition of stop solution, and absorbance is spectrophotometrically measured at 450 nm. A standard curve is prepared relating color intensity to the concentration of DHEA-S. The sensitivity of this ELISA test is 0.1 µg/ml.

II. Application:

Quantitative protein detection

III. Specificity:

Human DHEA-S.

IV. Sample Type:

- Serum and plasma

V. Kit Contents:

Components	K7428-100	Part No.
Plate coated with Goat anti-rabbit IgG	12 stripsx8 wells	K7428-100-1
Standard set: (0.25 ml)	7 vials	K7428-100-2.x*
DHEA-S Enzyme Reagent	6 ml	K7428-100-3
Anti-DHEA-S Ab Reagent	6 ml	K7428-100-4
Wash Concentrate (20X)	25 ml	K7428-100-5
TMB Substrate	12 ml	K7428-100-6
Stop Solution	12 ml	K7428-100-7

*x denotes Standard concentration vials: K7428-100-2.1, K7428-100-2.2, K7428-100-2.3, K7428-100-2.4, K7428-100-2.5, K7428-100-2.6, K7428-100-2.7

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** 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 (18-24° C).

VIII. Warning & Precautions:

- Potential biohazardous materials: The calibrator and control 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.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. 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.

1. Place the desired number of coated strips into the holder
2. Pipet 10 µl of each standards, control, and samples into designated wells.

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3. Add 50 μ l of DHEA-S Enzyme-Reagent into each well.
4. Add 50 μ l of Anti-DHEA-S Antibody-Reagent into each well. Mix plate for 10 sec. It is important to have complete mixing in this step.
5. Incubate for 60 min. at room temperature. Briskly shake out the contents of the wells.
6. Remove liquid from all wells & wash wells three times with 350 μ l of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 μ l of TMB substrate to all wells & incubate for 30 min. at room temperature.
8. Add 50 μ l of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 10 min. after adding the stopping solution.

- XI. Calculation:** Construct the standard curve; plot the absorbance for the DHEA-S standards (vertical axis) versus standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Value above the highest point of the standard are retested after diluting with "0" standard.

Example of a Standard Curve:

Standard (μ g/ml)	OD (450 nm)
Standard 1 (0)	2.02
Standard 2 (0.1)	1.34
Standard 3 (0.5)	0.95
Standard 4 (1)	0.70
Standard 5 (2.5)	0.40
Standard 6 (5)	0.24
Standard 7 (10)	0.13

Expected Values: It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

Classification	Normal Range (μ U/ml)
Male	1.0-4.2 μ g/ml
Female	
Premenopausal	0.8-3.9 μ g/ml
Term Pregnancy	0.2-1.2 μ g/ml
Postmenopausal	0.1-0.6 μ g/ml
Newborn (both sexes)	1.7-3.6 μ g/ml

Conversion Factor: 1 μ g/ml = 2.6 μ mol/L

XII. RELATED PRODUCTS:

DHEA (2172)

FOR RESEARCH USE ONLY! Not to be used on humans.