



DHEA-S Coated Tubes

Direct ¹²⁵I-Radioimmunoassay kit for the quantitative determination of serum DHEA-S.



Cat. BL-02CT-100
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Summary and background of the test:

The most important plasma adrenal C19-androgens are Dehydroepiandrosterone Sulfate (DHEA-S), Dehydroepiandrosterone (DHEA) and Androstenedione. In normal young adults, DHEA-S circulates at a concentration of approximately ten times that of Cortisol, and at least hundred times that of DHEA, which makes it the steroid of greatest concentration in human plasma. The adrenal androgens are synthesized from cholesterol by adrenal cortex and can be metabolized to physiologically more potent androgens like testosterone and its 5-alpha reduced metabolite dihydrotestosterone. The increased secretion of adrenal androgens and the associated early signs of sexual maturation are called adrenarche. Adults with hyperprolactinemia have increased secretion of androgens. Moreover, pituitary gonadotropins do not appear to be involved in the production of adrenal androgens. It appears, therefore, that the prolactin and ACTH control adrenal androgens secretion even if most studies suggest that DHEA and, to a lesser extent, androstenedione, are the adrenal androgens most responsive to ACTH stimulation. During pregnancy, since formation of estrogen in the placenta is dependent on circulating C19-steroids, it is possible to evaluate the placental clearance of maternal plasma DHEA-S through placental estradiol formation. The determination of DHEA-S should be considered in:

- premature adrenarche
- congenital adrenal hyperplasia
- Cushing's syndrome
- hirsutism

DHEA-S determination can be considered as a useful marker in the follow-up of hyperandrogenic women.

It should also be kept in mind that age changes and sex differences can modify serum DHEA-S concentrations throughout adulthood.

Principle of the test :

Radioimmunoassay is based on the ability of a limited quantity of antibody to bind a fixed amount of radiolabelled antigen (¹²⁵I-Ag). The percentage of bound radiolabelled antigen is inversely related to the increasing concentration of unlabelled analyte in the sample. Separation of the bound and free radiolabelled antigen is necessary in order to determine the quantity of unlabelled antigen. The Bio-Line DHEA-S kit utilizes the coated tubes methodology. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the remaining radioactivity in the coated tubes with data established using known standards in the same assay system.

Precautions:

1. Radioactive material: Radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, or hospitals for "In-Vitro" clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals.

Compliance with these basic rules of radiation safety should provide adequate protection:

1. Do not eat, drink, smoke, or apply cosmetics in areas where radioactive material is used.
2. Do not pipet by mouth reagents containing radioactive materials.
3. Wear protective clothing; i.e., lab coats and disposable gloves, in order to avoid direct contact with radioactive reagents.
4. Work with radioactive materials should be performed in a designed area.
5. Radioactive materials should be stored in an acceptable location.
6. A log should be kept for receipt and disposal of radioactive materials.
7. Radioactive spills or accidents should be taken care of immediately according to established procedures.
8. Disposal of radioactive materials must comply with prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

2. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.

3. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

Materials provided:

Kit contains sufficient reagents for 100 determinations:

1. **DHEA-S human serum based standards & control:** 8 vials containing each 500 µl except Zero 1ml. Control: 1.8 ± 0.4 µg/ml.
Standards: 0-0.1-0.5-1-2-4-8 µg/ml.
2. **¹²⁵I-DHEA-S tracer:** 1 vial (red solution) containing 52 ml. Activity < 5µCi or 185 kBq.
3. **Coated tubes:** 2 x 50 tubes, coated with Anti-DHEA-S antiserum (Rabbit).
4. **Wash solution concentrate:** 1 vial of 2 ml of concentrate, to be diluted into 250 ml NaCl 9‰ and stored at 4°C.

Reagents provided should be stored at 2° - 8° C.

Refer to the expiration date on the kit label for stability.

Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Gamma counter.
3. Logit log graph paper.
4. Horizontal shaker recommended (type IKA-VIBRAX-VXR), but a rotator could be used.
5. Test tube racks.
6. Vortex mixer.
7. 9‰ NaCl saline solution.

Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

Assay procedure:

Bring reagents to room temperature and mix before use. Label tubes for total counts (Tc), standards, control sera and unknowns.

1. Pipet 20 µl of standards, samples and controls into the corresponding tubes.
2. Add 500 µl of tracer solution (red) to each tube. Mix well (sideways shaking of whole rack).
3. Mix well, cover and incubate 45 minutes at 37°C.
4. Aspirate (or decant). Wash twice, adding 1 ml of wash solution to each tube, except Tc. Aspirate or decant.
5. Record the counts per minute (cpm) for each tube. Count all tubes for one minute.

DHEA-S Coated Tubes Flow chart

Tubes Reagents	Tc	B0	Stds. , Control	samples
Standards or samples (µl)	-	20	20	20
Tracer (µl)	500	500	500	500
Mix well (sideways shaking of the whole rack) and incubate 45 min at 37°C				
Wash solution	-	2 x 1 ml		
Aspirate or decant. Count 1 min.				

Data table (example)

Tube	Duplicate cpm		Mean cpm	%B/B ₀	Conc.ng/ml
Tc	50 566	50 572	50 569	-	
Zero	14 089	13 642	13 865	100 %	
100	12 879	12 639	12 759	92.0 %	
500	9 983	9 408	9 695	69.9 %	
1000	8 297	8 213	8 255	59.5 %	
2000	6 880	6 970	6 925	49.9 %	
4000	5 464	5 419	5 441	39.2 %	
8000	3 695	3 931	3 814	27.5 %	
Control	6 800	7 076	6 932	50.1 %	1977

Calculation of results:

Determine the average counts for each set of duplicate tubes. Divide this value by the average net counts of the B₀, and multiply by 100 to yield the % B/B₀

$$\% \text{ B/B}_0 = \frac{\text{cpm (Stds, Controls or unknowns)}}{\text{cpm (B}_0\text{)}} \times 100$$

Plot % B/B₀ for each standard vs its concentration in ng/ml on semi-log graph paper. The concentration of DHEA-S in the unknown samples may be read directly from the standard curve.

Expected Values:

	Range (µg/ml)
newborn	1600-3690
adult male:	
-Prepubescent	110-620
-24-40 years	1910-3380
-65-85 years	170-910
adult female	
- Prepubescent	150-650
-Adult	780-3430
-Term Pregnancy	230-1220
-Postmenoposal	120-660

Each laboratory should analyze normal samples to establish its own normal ranges.

Conversion factor: 1 ng/ml = 2.6 nmol/l

Specific performance characteristics:

1. Specificity:

The relative percent of cross-reactivity by weight of DHEA-S and various related compounds was evaluated for the antibody used in this assay. Cross-reactivities are expressed as the amount of DHEA-S required to reduce the binding of ¹²⁵I-DHEA-S by 50%, relative to the amount of a related compound required to do the same.

$$\text{Cross-reactivity of } x = 100 \times \frac{\text{conc. DHEA-S at 50\% B/B}_0}{\text{conc. compound } x}$$

Compound x	Cross-reactivity (%)
DHEA-S	100 %
DHEA	< 5 %
Progesterone	< 3 %
Estradiol	< 0.50 %
Cortisol	< 0.50 %
Testosterone	< 0.50 %

Estrone	< 0.50 %
17-OH-Progesterone	< 0.05 %
17-OH-Pregnenolone	<0.05 %
Corticosterone	< 0.05 %
Dihydrotestosterone	< 0.05 %
Androstenedione	< 0.05 %

2. Sensitivity:

The lowest detectable concentration of DHEA-S that can be reliably distinguished from zero with this kit has been evaluated to be ≤ 15 ng/ml.

3. Precision and reproducibility:

Assays variations of two serum samples are mentioned in the following table.

	Sample 1	Sample 2
Mean	783 μ g/ml	3613 μ g/ml
Within assay variation	6.6 %	5.7 %
Between assay variation	7.1 %	6.9 %

4. Linearity:

The results obtained when diluting a serum with elevated DHEA-S concentration with a DHEA-S- free serum are summarized in the following table

Dilution factor	Expected values	Experimental values
1:1	3602 μ g/ml	-
1:2	1801 μ g/ml	1755 μ g/ml
1:4	901 μ g/ml	897 μ g/ml
1:8	450 μ g/ml	439 μ g/ml
1:16	225 μ g/ml	217 μ g/ml
1:32	113 μ g/ml	106 μ g/ml

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