



1. NAME AND INTENDED USE

DHEAS-CT is a radioimmunoassay coated tube kit for the direct quantitative determination of dehydroepiandrosterone sulphate (DHEAS, DHEA-S04) in human serum or plasma.

2. INTRODUCTION

Dehydroepiandrosterone-sulphate is the major adrenal C-19 steroid. It is almost entirely secreted by the adrenal cortex. Because of its slow metabolism, there is none circadian variation of the serum concentration. Moreover, DHEAS level is 200 to 1000 times higher than that of DHEA and 20 times higher than of other steroid hormones. The secretion of DHEAS is stimulated by the ACTH, it circulates bound to albumin. DHEAS level is high at birth, low in childhood. It increases through puberty and gradually declines after the age of 25-30.

DHEAS has a weak activity but its metabolites such as testosterone and delta-4-androstenedione are strongly androgenic. DHEAS measurement is very helpful in the identification of the origin of the hyperandrogenism. It is of value in the assessment of premature adrenarche or delayed puberty. In adults, elevated concentrations of DHEAS indicate the presence of adrenal hyperplasia (especially those due to 11-hydroxylase defects or excessive ACTH stimulation) or an androgen secreting adrenal tumour.

High DHEAS levels are often found in the polycystic ovary syndrome (POC). The resulting hyperandrogenism leads to varying degrees of virilization.

Recently, the interest of DHEAS increased because of its potential anti-ageing effects and its role in Alzheimer's disease.

3. PRINCIPLE

The principle of the assay is based on the competition between the labelled DHEAS and DHEAS contained in standards or specimens to be assayed against a fixed and limited number of antibody binding sites bound to the solid phase (coated tubes).

After incubation, the unbound tracer is easily removed by a washing step.

The amount of labelled DHEAS bound to the antibody is inversely related to the amount of unlabelled DHEAS present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
COATED TUBES : ready to use. Polyclonal rabbit anti-DHEAS antibodies coated to the bottom of the tube.	100 tubes	2-8°C until the expiry date. Unused coated tubes removed from their bags should be stored in the original bag.
¹²⁵I -DHEAS : ready to use. ¹²⁵ I labelled DHEAS, phosphate buffer, bovine albumin, red dye, and sodium azide. ≤ 200 KBq (≤ 5.4 µCi).	1 55 ml vial	2-8°C until the expiry date.
STANDARDS 0 to 5 : freeze dried. Synthetic DHEAS, human serum, Kathon and sodium azide. 0 - 0.1 - 0.5 - 2 - 10 and 30 µmol/l (*)	6 vials qs 0.5 ml distilled water	2-8°C until the expiry date. 2-8°C 8 weeks after reconstitution.

(*) Standards are prepared gravimetrically.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbial contamination of the reagents or of the water used for washing.
Fully respect the incubation times and the washing instructions.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed on sera or plasma (heparin or EDTA). Do not use citrate plasma samples. Serum bilirubin concentrations $\leq 350 \mu\text{mol/l}$ or haemoglobin concentration up to 5 g/l has no effect on the measured DHEAS concentrations. Hyperlipemic samples should not be used. If the test is to be carried out within 1 week, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed and must be thawed only just before using. Do not refreeze samples for later use.

Dilution

If elevated DHEAS levels are suspected, standard 0 should be used for dilution (up to 1:20). It is recommended that disposable plastic tubes be used when carrying out the dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 25 μl and 500 μl . Their calibrations should be checked regularly.
Reagent dispenser 1 ml (for washing)
Distilled water.
Vortex type mixer.
Absorbent paper.
Water bath (37°C) .
Parafilm (optional).
Disposable plastic test-tubes.
Gamma scintillation counter calibrated for 125 Iodine.

7.2. Reconstitution of the standards

Reconstitute the standards with 0.5 ml of distilled water. Recap the vial. Mix gently by inversion to assure complete dissolution of the freeze-dried material.
N.B. : The reconstituted standards should stand at least 60 minutes after reconstitution before proceeding.

7.3. Protocol

All reagents should be brought to room temperature (18-25°C) at least 30 minutes before their use (one hour for the standards after reconstitution). Dispensing of reagents is also carried out at room temperature.

The assay requires the following groups of tubes :

T group, for the total activity determination.
Standard group, to establish the standard curve.
Reference group for the external controls.
Sx group, for the test samples.

It is recommended to perform the assay in duplicate for the standard groups, controls and samples.
Strictly observe the order in which reagents should be added.

Dispense 25 μl of standards, controls and samples to be assayed into the correspondingly-labelled coated tubes.

Add 500 μl of ^{125}I -DHEAS to each tube (and T group).

Mix each tube gently with a Vortex-type mixer.

Incubate 1 hour at 37°C in a water bath after covering the tubes with plastic film.

Decant liquid from each assay tube and **tap** the head of each tube firmly against absorbent paper (except T tubes).

Wash once with 1 ml of distilled water (except T tubes), shaking the rack by hand.

Empty the tubes and tapping firmly against absorbent paper (except T tubes). Let the tubes stand upside down at least 5 min.

Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter, at least one minute.

8. QUALITY CONTROL

Good laboratory practices require the use of quality control samples in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes compute the mean counts. Calculate B/Bo values. Draw up the standard curve on semi-log graph by plotting the B/Bo of the standards against their concentrations. Read sample values directly from the standard curve, and correct the read value for the dilution factor, if necessary.

These data must not be substituted for results obtained in the laboratory.

Conversion to pg/ml may be accomplished by using the following equation :

$$\text{DHEAS } (\mu\text{g/ml}) = \text{DHEAS } (\mu\text{mol/l}) \times 0.3685$$

Typical standard curve (example of result) : these data must not be substituted for results obtained in the laboratory.

Groups of tubes	CPM Mean	B/Bo x 100	Concentration $\mu\text{mol/l}$
T	58830	-	
Standard 0 0 $\mu\text{mol/l}$	47400	100	
Standard 1 0.1 $\mu\text{mol/l}$	41777	88.1	
Standard 2 0.5 $\mu\text{mol/l}$	35711	75.3	
Standard 3 2 $\mu\text{mol/l}$	28152	59.4	
Standard 4 10 $\mu\text{mol/l}$	17480	36.9	
Standard 5 30 $\mu\text{mol/l}$	10503	22.1	
Sample A	26838	45.6	2,5
Sample B	15536	26.5	13.4

10. PROCEDURAL LIMITATIONS

Strict adherence to the exact procedures described within this package insert and careful technique should be exercised to obtain reliable results with the DHEAS-CT kit.

11. EXPECTED VALUES

Each laboratory must establish its own range of normal values.

Serum DHEAS values were measured in 289 samples. Results are shown in table below :

	n	Age	Mean ($\mu\text{mol/l}$)	Limits
Women	152	21-50	4.85	0.47 - 11.6
Men	137	21-50	7.44	1.99 - 17.0

The expected values, age related, are shown in the following table :

AGE Year	WOMEN			MEN		
	n	Mean (mol/l)	Range ($\mu\text{mol/l}$)	n	Mean ($\mu\text{mol/l}$)	Range ($\mu\text{mol/l}$)
< 10	29	1.37	0.06-7.72	14	0.84	0.08-4.06
11-15	45	4.62	1.51-10.6	11	2.97	1.22-4.69
16-20	34	5.27	1.39-11.5	14	5.29	1.84-7.93
21-30	52	5.17	1.87-10.8	49	8.85	3.24-14.3
31-40	50	4.64	1.68-9.67	28	8.26	1.99-13.7
41-50	50	4.74	0.51-10.5	60	5.9	2.32-11.2
51-60	34	2.62	0.97-5.47	59	4.6	1.03-8.53
61-70	53	2.05	0.62-4.74	-	-	-

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Precision

This was evaluated with 4 samples with different concentrations assayed 14 times in the same series or 4 other samples twice in 11 different series.

Samples	Within-run	
	Mean value ($\mu\text{mol/l}$)	CV (%)
1	0.58	5.5
2	2.86	6.5
3	8.71	5.6
4	25.5	3.5

Samples	Between-run	
	Mean value ($\mu\text{mol/l}$)	CV (%)
5	0.68	8.1
6	2.69	6.4
7	6.58	4.8
8	19.8	4

12.2. Recovery test

Known quantities of DHEAS were added into different serum pools. The recovery percentage of DHEAS obtained was in the range of 88 % and 114 % with a mean value of 103 %.

12.3. Specificity

Determined from equivalent displacement measurements at 50 % binding. The antiserum used in the test shows the following cross-reactions :

Compound	Cross reaction (%)	Compound	Cross reaction (%)
DHEAS	100	DHEA glucuronide	nd
Aldosterone	nd	5 α -Dihydrotestosterone	nd
Androstendiol	nd	Estradiol	nd
Androstendione	0,01	Cyproterone acetate	nd
Androsterone	nd	Estrone	nd
Metyrapone	nd	Estrone-3-sulphate	1,6
Androsterone glucuronide	nd	17 α -Hydroxyprogesterone	nd
Androsterone sulphate	0,3	19-Hydroxy-4-androstene-3,17-dione	nd
Corticosterone	nd	Pregnenolone	nd
Cortisol	nd	Progesterone	nd
Dexamethasone	nd	Testosterone	nd
DHEA	nd		

nd = non detectable

12.4. Detection limit

The detection limit is defined as being the smallest concentration, different from the zero, with a probability of 95 %. It has been assessed as 0.03 μ mol/l.

ASSAY FLOW-CHART

TUBES	Standards Controls Samples μ l	125 μ DHEAS μ l		Distilled water μ l	
T	-	500	Mix ----	-	Count
Standards	25	500	Incubate 1h at 37°C ----	1000	Empty the tubes -----
Controls and samples	25	500	Empty the Tubes	1000	Count