RK-620CTACE140224

DHEA-SO4 [^{125}I] RIA KIT

(Ref**:** RK-620CT)

Description

The DHEA-SO_4[^{125}I] assay system provides a direct quantitative *in vitro* determination of DHEA-SO_4 in human serum or plasma. DHEA-SO_4 can be assayed in the range of 0-30 µmol/L (0-11.05 µg/mL). Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and the assay of 43 samples in duplicate.

Introduction

Dehydroepiandrosterone sulphate (DHEA-SO_4) is almost exclusively synthesized by the adrenal cortex, and it is the most abundant steroid hormone in the peripheral circulation. It is the main source of the urinary 17-ketosteroids. The metabolic clearance of DHEA-SO_4 is slow, and it is converted mostly to oestrogens. The hormone has a maximum level from puberty until 20-30 years of age, then there is a gradual decrease in the blood DHEA-SO_4 concentration mainly in the menopause of women. Although the physiological role of DHEA-SO_4 is not well established the serum level of this steroid hormone has an informative pathophysiological value.

1. The serum DHEA-SO_4 radioimmunoassay seems to be a reliable tool to assess adrenal androgen function and the glandular overproduction of androgens.
2. High DHEA-SO_4 values indicate a virilizing disorder of adrenal origin in women. This includes mainly adrenal neoplasms or early or late onset of congenital adrenal hyperplasia.
3. Monitoring the DHEA-SO_4 concentration may be useful to control the adrenal suppressive therapy (dexamethasone).
4. Low DHEA-SO_4 levels can be an indicator of hormone-dependent immunological disorders.
5. Low levels of DHEA-SO_4 may be related to the development of diseases that increases with age such as cancer and atherosclerosis. In these circumstances a systematically repeated assessment of the blood DHEA-SO_4 values is recommended.

Principle of the method

This assay is based on the competition between unlabelled DHEA-SO_4 and a fixed quantity of ^{125}I-labelled DHEA-SO_4 for a limited number of binding sites on DHEA-SO_4 specific antibody. During incubation the antigen-antibody immuno-complex is immobilized on the reactive surface of test tubes, afterwards the reaction mixture is discarded and the radioactivity measured in a gamma counter. The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against a series of calibrators containing known amount of DHEA-SO_4, a calibration curve is constructed, from which the unknown concentration of DHEA-SO_4 in patient samples can be determined.

Contents of the kit

1 bottle	^{125}I-TRACER, Ready to use. 55 ml per vial, containing less than 275 kBq DHEA [^{125}I] in buffer with 0.1% NaN_3.
6 vials	STANDARDS, Ready to use. 0.5 ml per vial, containing 0, 0.3, 1, 3, 10, 30 µmol/L in human serum with 0.1% NaN_3.
1 bottle	ANTISERUM, Ready to use. 55 ml per vial, containing polyclonal anti-DHEA-SO_4 (rabbit) IgG in buffer with 0.1 % NaN_3.
1 vial	CONTROL SERUM. Lyophilised human serum with 0.1% NaN_3. The concentration of the serum is specified in the quality certificate enclosed.
2 boxes	COATED TUBES, 2x50 pieces of 12x75 mm RIA tubes, packed in plastic boxes.
1 pc	Quality certificate.
1 pc	Pack leaflet.

Materials and equipment required

Test tube rack allowing fixing of tubes
Precision pipettes (10 and 500 µl)
Plastic film to cover tubes
Vortex mixer
Shaker (orbital or horizontal)
Absorbent tisue
Gamma counter

Recommended tools and equipment

Repeating pipettes

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice.

Sera can be stored at 2-8°C for two days after collection. For later analysis they should be stored deep-frozen. Repeated freezing and thawing should be avoided.

Do not use lipemic, hemolyzed or turbid specimens.

Preparation of reagents, storage

Store the reagents between 2 and 8°C. At this temperature each reagent is stable until expiry date.

Add 500 µl distilled water to the lyophilised control serum. Mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes.

Equilibrate all reagents and serum samples to room temperature (min. for an hour). Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide refer to Table 1)

1. Label coated tubes in duplicate for each standard (S1-S6), control serum (C) and samples (Sx). Optionally, label two uncoated test tubes for total count (T).
2. Pipette 10 µl of standards, control and samples into the properly labelled tubes.
3. Pipette 500 µl of tracer into each tube.
4. Pipette 500 µl antiserum into each tube except T.
5. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm recommended).
6. Incubate tubes for 1 hour at room temperature.
7. Aspirate or decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 5 minutes.
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the DHEA-SO_4 concentrations of the samples as described in calculation of results.

Table 1. Assay Protocol (all volumes in microliters) T=total count, S_{1-6}=standards, S_X=sample, C=control

Tubes	T	S_{1-6}	C	S_X
Standard		10		
Control			10	
Sample				10
Tracer	500	500	500	500
Antiserum		500	500	500
Shake for 1 hour at room temperature				
Decant the fluid and blot on filter paper				
Count all tubes				

Calculation of results

Calculate the average counts per minute (CPM) for each pair of assay tubes.

Calculate the percent B_0/T for zero standard (S_1) by using the following equation:

$$B_0/T\ \% = 100 * S_1(cpm) / T(cpm)$$

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0\% = 100 * S_{2\text{-}6}\ ;\ C\ ;\ S_x(cpm) / S_1(cpm)$$

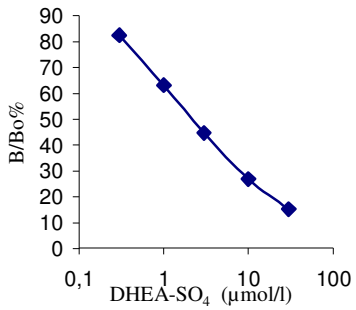
For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 3% of total count.

Using semi-logarithmic graph paper plot B/B_O% for each standard versus the corresponding concentration of DHEA-SO_4. Figure 1 shows a typical standard curve. Determine the DHEA-SO_4 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Automated data processing systems are also available.

Table 2. Typical Assay Data

Tubes	Counts CPM1	Counts CPM2	AVG CPM	B/T %	B/Bo %
T	79631	80972	80301		
S1	69071	69564	69317	86.3	100
S2	57468	56881	57174	71.2	82.5
S3	43593	44028	43810	54.6	63.2
S4	31210	30921	31065	38.7	44.8
S5	18960	18413	18686	23.3	26.9
S6	10912	10280	10596	13.2	15.3
C	24014	24225	24119	30.0	34.8

Figure 1.
A typical standard curve
(Do not use to calculate sample values)



Conversion of SI units can be performed according to the following formula:
1 µmol/L = 0.37 µg/ml
1 µg/ml = 2.71 µmol/L

Characterization of the assay

Sensitivity

Limit of detection: 0.064 µmol/L.

Specificity

Different endogen hormones were added to the "0" standard at two concentration levels (A=70 nmol/L, B=700 nmol/L). The appaerant DHEA-SO_4 concentrations measured in these experients are reported in Table 3.

b: below detection limit (0.064 µmol/L)

Table 3. (concentration in µmol/L)

Analyte	A	B	Analyte	A	B
Aldosterone	b	b	Cortisol	b	b
Androtenedione	b	0.1	Estriol	b	b
5α-Dihydro-testosterone	b	b	Estrone	b	b
5β-Dihydro-testosterone	b	b	Progesterone	b	b
Androstanediol	b	b	Pregnenolone	b	b
17α-hydroxi-progesterone	b	b	Testosterone	b	b
Androstenediol	b	b	DHEA	0.15	2.29

Hence the physiological concentration of DHEA is around 13-24 nmol/L (literature data) the distortion due to the crossreactivity is negligible.

Precision, reproducibility

Six control samples were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 22 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (µmol/L)	CV%	Mean (µmol/L)	CV%
0.8	3.05	0.78	5.32
1.96	2.91	1.78	3.9
3.78	4.61	3.91	5.8
5.46	1.63	5.66	3.66
14.25	2.28	13.4	3.59
18.27	3.42	19.38	5.01

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of DHEA-SO_4. The average per cent recovery for 5 serum samples spiked with DHEA-SO_4 at 3 levels was: 103.5% (96.8% – 108.6%).

Dilution test (linearity)

6 samples were serially diluted with zero-standard and measured according to kit protocol. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$y = 0.9854x - 0.0613$ $R^2 = 0.9988$ n = 30

Expected reference values

It is recommended that each laboratory establish its own reference intervals. Concentration values are expressed in µmol/L.

Male			
N	Age group (years)	Median	Range (central 95%)
100	18 - 30	10.1	3.8 - 17.5
100	31 - 50	6.7	3.0 - 14.2
50	51 - 60	5.2	1.6 - 11.3
27	> 60	2.1	0.7 - 8.1

Female			
N	Age group (years)	Median	Range (central 95%)
100	18 - 30	6.22	2.1 - 12.8
100	31 - 50	4.89	1.5 - 11.5
50	51 - 60	2.95	0.5 - 8.9
17	> 60	2.75	0.4 - 5.9

The results obtained should only be interpreted in the context of the overall clinical picture. None of the in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Source of error!** Do not use a shaker where some tubes can be exposed to heating. Do not place the shaker directly by an air conditioning or heating device or by an open window. Any differences in temperature between tubes during incubation can lead to serious measuring errors.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

Radioactivity

This kit contains radioactive material. Receipt, acquisition, possession, or use of radioactive materials are subject to regulations, and a licence of (inter)national authorizing bodies. It is the responsibility of the user to ensure that local regulations or codes of practice are satisfied.

Potentially infectious materials

Human blood products provided as components of this product have been obtained from donors tested individually and found negative for Human Immunodeficiency Virus antibody (HIV-Ab), Hepatitis B surface Antigen (HBsAg), Hepatitis-C antibody and Treponema antibody, using approved EIA methods. Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious material*.

Chemical hazard

Some components contain sodium azide as an Antimicrobial Agent. Dispose the waste by flushing it with copious amounts of water to avoid build up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 115 mg.

	Use by date	LOT	Batch code
2 8°	Storage temperature	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device	AS	Antiserum
	Manufacturer	REF	Catalogue number
	Radioactive material		

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