

**REF**

GD7255 00

# DHEA-S

Enzyme-immunoassay for the quantitative  
determination of Dehydroepiandrosterone sulphate  
in serum or plasma

**IVD**

## INDICATION

Dehydroepiandrosterone (DHEA) is an androgen with a MW of 288.4 Dalton secreted in adrenals. The main derivate of DHEA present in human tissue is DHEA sulphate (DHEA-S). Since birth, DHEA-S serum concentration is increasing continuously showing a pronounced peak after puberty and maximal levels at the age of 20. After that, serum DHEA-S level continuously decreases. As DHEA-S is the main component of 17-ketosteroids in serum, this test may substitute for column tests for determination of 17-ketosteroids in urine.

Elevated DHEA-S concentrations are found in adrenogenital syndrome, hirsutism, acne, benign hyperplasia of adrenals and adrenal tumors, Stein-Leventhal syndrome, polycystic ovary syndrome.

Decreased levels of DHEA-S are found in hyperlipidemia, psychotic states, psoriasis, adrenal insufficiency.

## PRINCIPLE OF THE ASSAY

The present test is a competitive "one step" immunoassay. Tested specimen is placed into the microwells coated by specific anti-DHEA-S antibodies simultaneously with DHEA-S conjugated to horseradish peroxidase (HRP). DHEA-S from the specimen competes with the conjugated antigen for antibodies coated on the solid phase. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture. The developed colour density, revealed at 450 nm, is inversely related to the quantity of DHEA-S present in the specimen.

## KIT CONTENT

- 1. Reagent A – Microplate**  
12x8 strips.  
8 wells breakable strips, coated with antibodies anti-DHEA-S. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.
- 2. Reagent B – Enzymatic Tracer**  
1 vial of 11 ml.  
Ready to use liquid reagent containing DHEA-S conjugated to Horseradish peroxidase (HRP).
- 3. Reagent C – Washing Solution 21x**  
1 vial of 22 ml.  
Concentrated solution to be diluted 1:21 with distilled water. It contains a Phosphate buffer, a detergent and preservatives (0.1% Kathon GC).
- 4. Reagent D/E – Chromogen/Substrate**  
1 vial of 11 ml.  
Ready to use solution containing Tetramethylbenzidine (TMB) with activators and stabilizers.  
**Avoid light exposure.**
- 5. Reagent F – Stop Solution**  
1 vial of 11 ml.  
Ready to use solution containing a solution of Sulphuric acid.  
**Avoid any skin contact.**
- 6. DHEA-S Standards**  
6 vials of 0.8 ml each.  
Ready to use liquids containing DHEA-S at the following concentrations:  
**S<sub>0</sub>: 0 µg/ml, S<sub>1</sub>: 0.1 µg/ml, S<sub>2</sub>: 0.3 µg/ml, S<sub>3</sub>: 1 µg/ml, S<sub>4</sub>: 3 µg/ml, S<sub>5</sub>: 10 µg/ml \***
- 7. DHEA Control**  
1 vial of 0.8 ml.  
Ready to use liquid reagent containing DHEA-S. Concentration range is stated on the label.
- 8. Cardboard sealers**  
2 cardboard sealers to be used to cover the plate during the incubations.
- 9. Package insert:** instruction for use GD7255 00 it/ing.

\* Alternative units: **1 µg/ml = 2.60 µmol/l**

## MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

## STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

## AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Microplate incubator 37 ± 1 °C.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

## SAMPLES

Serum or plasma (in ACD heparin). Specimens can be stored up to 48 hours at 2-8 °C before testing; for a long storage, the specimens should be frozen at -20 °C. Repeated freezing/thawing should be avoided. Turbid, hemolytic, lipemic, or contaminated microbiologically samples should be avoided.

## REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:21 with distilled or ELISA grade water (e.g.: 1 ml of Reagent C + 20 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 5 days at room temperature or 30 days at +2-8 °C. It is recommended to store diluted washing solution at room temperature for immediate use.

## WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

## ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blinking" of the instrument is to be carried out in the Standard 0 U/ml.

### ASSAY SCHEME

- Put the desired number of microstrips into the frame.
- Follow the scheme:

	Microwells coated with antibody anti-DHEA-S		
	REAGENTS	Standard/Control	Sample
Immunological reaction	Standard/Control	25 µl	-
	Sample	-	25 µl
	Reagent B (Enzymatic Tracer)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate <b>60 minutes</b> at <b>37 (± 1) °C</b>		
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid		
Colorimetric reaction	Reagent D (Chromogen-Substrate)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate <b>15 minutes</b> at <b>18-25 °C, avoiding light exposure</b>		
	Reagent F (Stop Solution)	100 µl	100 µl
	Read the absorbance of each well against air at 450 nm (and 620-630 nm)		

### CALCULATION OF RESULTS

- Calculate the mean value of the OD 450 nm obtained for each duplicate of standard, control and samples.
- Draw a standard curve by plotting the absorbances of the standards with the corresponding concentrations. A lin-log method for data reduction is recommended. Alternatively, the calculation system of the microplate reader software can be used.
- Calculate the concentrations of control and samples by interpolation from the obtained calibration curve.

### QUALITY CONTROL

DHEA-S control concentration should fit into the established range stated on the label.

### EXPECTED VALUES

Based on data obtained by Minias Globe Diagnostics, the reference ranges in the following table are suggested. However, it is recommended that each laboratory establishes its own reference range.

Sex, age	Range	
	µg/ml	µmol/l
<b>Males</b>		
newborn	1.08 - 4.06	2.81 - 10.56
1 month-5 years	0.01 - 0.41	0.03 - 1.07
6-9 years	0.03 - 1.45	0.07 - 3.77
10-11 years	0.12 - 1.15	0.31 - 2.99
12-17 years	0.20 - 5.55	0.52 - 14.43
18-30 years	1.25 - 6.19	3.25 - 16.09
31-60 years	0.59 - 4.52	1.53 - 11.75
51-60 years	0.20 - 4.13	0.52 - 10.74
> 61 years	0.10 - 2.35	0.26 - 6.11
<b>Females</b>		
newborn	0.10 - 2.48	0.26 - 6.45
1 month-5 years	0.05 - 0.55	0.13 - 1.43
6-9 years	0.03 - 1.40	0.07 - 3.64
10-11 years	0.15 - 2.60	0.39 - 6.76
12-17 years	0.20 - 5.35	0.52 - 13.91
18-30 years	0.29 - 7.81	0.75 - 20.31
31-60 years	0.12 - 3.79	0.31 - 9.85
post menopausal	0.30 - 2.60	0.78 - 6.76
pregnancy		
1 <sup>st</sup> trimester	0.38 - 3.60	0.99 - 9.36
2 <sup>nd</sup> trimester	0.42 - 3.00	1.09 - 7.80
3 <sup>rd</sup> trimester	0.32 - 2.50	0.83 - 6.50

**ANALYTICAL PERFORMANCES****Sensitivity**

The lowest detectable concentration of DEHEA-S is 0.1 µg/ml.

**Precision**

It has been calculated on Controls tested in replicates, in different days. CV% between 4.2 - 14% have been obtained, depending on OD 450 nm value.

**Correlation**

The present kit well correlates with similar ones in commerce.

**PRECAUTIONS IN USE**

The reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**REFERENCES**

1. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
2. E. Friess, et al., Eur J Clin Invest, (2000) 30 Suppl 3:46-50.
3. C. Longscope, J Endocrinology, (1996) 150 Suppl: S125-S127.
4. J. Herbert, Lancet, (1995) 345:1193-1194.
5. A. Michael, et al., Biol. Psychiatry, (2000) 48(10): 989-95.
6. C.R. Dequet and D.J. Wallace, Current Opin Invest Drugs, (2001) 8: 1045-53.
7. W.M. Jeffries., Med Hypotheses, (1998) 51(2):111-4.
8. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.