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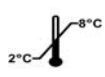
Instructions for use

DHEA ELISA

REF AA E-1600R



RUO

For research use only – Not for use in diagnostic procedures

DHEA ELISA

INTRODUCTION

Intended Use

The **DHEA ELISA** is a competitive immunoassay for the quantitative measurement of Dehydroepiandrosterone (DHEA) in serum and plasma.

PRINCIPLE

The DHEA ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with an anti-DHEA antibody. An unknown amount of DHEA present in the sample competes with an DHEA-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of DHEA in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of DHEA in the sample.

WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

REAGENTS

Reagents provided

96 **AA E-1631 Microtiterplate**

12x8 (break apart) strips with 96 wells; Wells coated with anti-DHEA antibody.

Standards

ready to use

	Cat. no.	Standard	Concentration	Volume / Vial
STANDARD A	**AA E-1601**	Standard A (0)	0 ng/ml	0.6 ml
STANDARD B	**AA E-1602**	Standard B (1)	0.3 ng/ml	0.6 ml
STANDARD C	**AA E-1603**	Standard C (2)	1 ng/ml	0.6 ml
STANDARD D	**AA E-1604**	Standard D (3)	3 ng/ml	0.6 ml
STANDARD E	**AA E-1605**	Standard E (4)	10 ng/ml	0.6 ml
STANDARD F	**AA E-1606**	Standard F (5)	30 ng/ml	0.6 ml

CONTROL 1 + CONTROL 2 **AA E-1651 + AA E-1652 Control low / Control high**

2 vials, 0.6 ml each, ready to use; containing DHEA in serum.

For control values and ranges please refer QC-Datasheet

CONJUGATE **AA E-1640 Enzyme Conjugate**

1 vial, 13 ml, ready to use; horseradish peroxidase labeled DHEA in buffered matrix.

SUBSTRATE **AA E-1655 Substrate Solution**

1 vial, 26 ml each, ready to use; contains Tetramethylbenzidine (TMB)

Hazards identification:

H360D May damage the unborn child.

STOP-SOLN **AA E-1680 Stop Solution**

1 vial, 9 ml, ready to use; contains 2 N hydrochloric acid solution

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

WASH-CONC 10x **AR E-0030 Wash Solution**

2 vials, each 50 ml (10X concentrated);

see "Reagent Preparation".

Note: Additional Standard A for sample dilution is available upon request.

Materials required but not provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes (25 µL, 100 µL, 200 µL, 300 µL).
- Microplate mixer operating more than 600 rpm
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

Storage conditions

When stored at 2°C to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. After first opening the reagents are stable for 30 days if used and stored properly.
Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

Wash Solution:
Dilute 50 mL of 10X concentrated *Wash Solution* with 450 mL deionized water to a final volume of 500 mL.
The diluted Wash Solution is stable for at least 12 weeks at room temperature (21-26°C).

Disposal of the kits

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged test kits

In case of any severe damage of the test kit or components, the manufacturer have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN

For determination of DHEA **serum or plasma (EDTA)** can be used. The procedure calls for 25 µL sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain DHEA concentrations higher than the highest standard (30 ng/mL) should be diluted with Standard A before assay. The additional dilution step has to be taken into account for the calculation of the results. Do not use grossly haemolytic, icteric or grossly lipaemic specimens.

ASSAY PROCEDURE

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.

Assay procedure

Each run must include a standard curve.

1.	Prepare a sufficient number of microplate wells to accommodate standards and samples in duplicates.
2.	Dispense **25 µL** of each **Standard, Sample and Control** with new disposable tips into appropriate wells.
3.	Dispense **100 µL** of **Enzyme Conjugate** into each well.
4.	Incubate for **60 minutes** at room temperature on a plate shaker (> 600 rpm). **Important Note:** Optimal reaction in this assay is markedly dependent on shaking of the microplate!
5.	Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µL per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
6.	Add **200 µL** of **Substrate Solution** to each well.
7.	Incubate without shaking for **30 minutes** in the dark.
8.	Stop the reaction by adding **50 µL** of **Stop Solution** to each well.
9.	Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

Calculation of results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the package insert have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Example of typical standard curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Optical Units (450nm)
Standard A (0 ng/mL)	3.003
Standard B (0.3 ng/mL)	2.501
Standard C (1 ng/mL)	1.912
Standard D (3 ng/mL)	1.220
Standard E (10 ng/mL)	0.647
Standard F (30 ng/mL)	0.341

EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DHEA ELISA the following values are observed:

Population	Range
Adult Males	1.8 – 12.5 ng/mL
Adult Woman	1.3 – 9.8 ng/mL

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The lowest analytical detectable level of DHEA that can be distinguished from Standard A is 0.07 ng/mL at the 2SD confidence limit.

Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to DHEA.

Steroid	% Cross reaction
DHEA-S	< 0,01
Testosterone	< 0,01
5α-Dihydrotestosterone	< 0,01
Androstendione	0,06
Progesterone	0,23
17α-Hydroxyprogesterone	< 0,01
Pregnenolone	0,01
17-Hydroxy-Pregnenolone	0,07
Desoxycorticosterone	0,05
Corticosterone	< 0,01
Cortisol	< 0,01

Assay dynamic range

The range of the assay is between 0.3 – 30 ng/mL.

Reproducibility

Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

	Serum 1	Serum 2	Serum 3
Mean (ng/mL)	2.08	5.34	20.84
SD	0.16	0.39	1.511
CV (%)	7.9	7.3	7.3
n =	20	20	20

Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples in 11 different tests.

	Serum 1	Serum 2	Serum 3
Mean (ng/mL)	2.14	5.26	20.63
SD	0.15	0.27	1.03
CV (%)	6.9	5.1	5.0
n =	11	11	11

Recovery

Using the standard matrix a spiking solution of 1000 ng DHEA/mL was prepared. 500 µL of three sera were spiked with 1.5, 3 and 5 µL of the spiking solution leaving the serum matrices relatively intact. All samples were measured by the DHEA ELISA procedure.

Sample	Spiking (ng/mL)	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1	-	0	-	-
	3	2.63	3	88%
	6	5.52	6	92%
	10	10.04	10	100%
2	-	0.96	-	-
	3	3.36	3.96	85%
	6	5.67	6.96	81%
	10	8.73	10.96	80%
3	-	1.69	-	-
	3	4.05	4.69	86%
	6	7.11	7.69	92%
	10	10.24	11.69	88%

Linearity

Three serum samples were assayed undiluted and diluted with Standard A.

Serum	Dilution	Measured (ng/mL)	Expected (ng/mL)	Linearity (%)
1	-	11.34	./.	./.
	1 in 2	5.91	5.67	104%
	1 in 4	3.24	2.84	114%
	1 in 8	1.55	1.48	105%
2	-	4.78	./.	./.
	1 in 2	2.57	2.39	108%
	1 in 4	1.23	1.2	103%
	1 in 8	0.49	0.6	82%
3	-	12.08	./.	./.
	1 in 2	6.57	6.04	109%
	1 in 4	3.57	3.02	118%
	1 in 8	1.82	1.51	120%

LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Drug Interferences

Any medication (cream, oil, pill etc.) containing DHEA will significantly influence the measurement of this analyte.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!