

ab108669 – DHEA sulfate (DHEA-S) ELISA Kit

Instructions for Use

For the quantitative measurement of IgG class antibodies against DHEA sulfate (DHEA-S) in Human serum and plasma (citrate).

This product is for research use only and is not intended for diagnostic use.

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1. **BACKGROUND**

Abcam's DHEA sulfate (DHEA-S) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of DHEA sulfate in plasma and serum.

A 96-well plate has been precoated with anti-DHEA sulphate antibodies. Samples and the DHEA sulfate-HRP conjugate are added to the wells, where DHEA sulfate in the sample competes with the added DHEA sulfate-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of DHEA sulfate in the sample and the intensity is measured at 450 nm.

Dehydroepiandrosterone sulfate (DHEA-S), is a natural steroid hormone produced in the adrenal glands. DHEA-S is derived from the enzymatic conversion of DHEA in adrenal tissue, gonads, adipose tissue and the brain. It is the most abundant steroid hormone in the Human body and is the precursor of all sex steroids

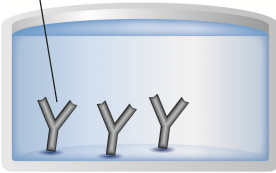
As most DHEA-S is produced by the zona reticularis of the adrenal gland, it is argued that it has a role in immunity and stress response. DHEA-S may have more biological roles. Its production in the brain suggests that it also has a role as neurosteroid.

Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism, while elevated levels occur in several conditions, e.g. virilising adrenal adenoma and carcinoma, 21-hydroxylase and 3 β -hydroxysteroid dehydrogenase deficiencies and in some cases of female hirsutism. Women with polycystic ovary syndrome tend to have normal or mildly elevated levels of DHEA-S. As very little DHEA-S is produced by the gonads, measurement of DHEA-S levels may aid in the localization of androgen source in virilising conditions.

DHEA-S shows no diurnal variation.

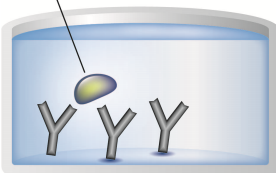
2. ASSAY SUMMARY

Capture Antibody



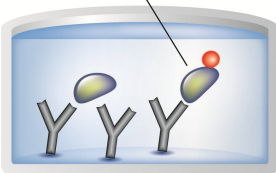
Prepare all reagents, samples, controls and standards as instructed.

Sample



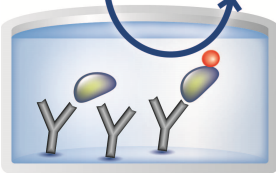
Add samples, standards and controls to wells used.

Labeled HRP-Conjugate



Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.

Substrate Colored Product



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-DHEA sulfate Antibody Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
DHEA sulfate-HRP Conjugate	12 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Serum Diluent	100 mL	2-8°C
Control A	1 mL	2-8°C
Control B	1 mL	2-8°C
DHEA sulfate Standard 0 – 0 µg/mL	1 mL	2-8°C
DHEA sulfate Standard 1 – 0.1 µg/mL	1 mL	2-8°C
DHEA sulfate Standard 2 – 0.4 µg/mL	1 mL	2-8°C
DHEA sulfate Standard 3 – 1.0 µg/mL	1 mL	2-8°C
DHEA sulfate Standard 4 – 4.0 µg/mL	1 mL	2-8°C
DHEA sulfate Standard 5 – 10.0 µg/mL	1 mL	2-8°C
10X Wash Solution	50 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells.

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **10X Wash Solution**

Dilute the solution with distilled water to a final volume of 500 mL. The diluted solution is stable for 30 days at 2-8°C.

- All other solutions are supplied ready to use.

10. SAMPLE COLLECTION AND STORAGE

- The determination of DHEA sulfate can be performed in plasma as well as in serum. Store the sample at -20° C if the determination is not performed on the same day of the sample collection.

Avoid repeated freezing and thawing

- Immediately before use, dispense 20 µL sample in 980 µL 1X Serum Diluent. Mix well.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 μ L to 350 μ L to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**
 - 12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.
 - 12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.3 Add 30 μ L standards, control or diluted samples into the respective wells. Add 100 μ L DHEA sulfate-HRP Conjugate to each well. Leave a well empty as the substrate blank (without standards, control or diluted sample) and do not add the conjugate to this.
 - 12.4 Cover wells with the foil supplied in the kit.
 - 12.5 Incubate for 1 hour at 37°C.
 - 12.6 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μ L diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 seconds. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.
 - 12.7 Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.
 - 12.8 Add 100 μ L TMB Substrate Solution into all wells.

- 12.9 Incubate for exactly 15 minutes at room temperature in the dark.
- 12.10 Add 100 μ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Agitate the microplate gently using a rotating mixer. Any blue color developed during the incubation turns into yellow.
- 12.11 Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution.

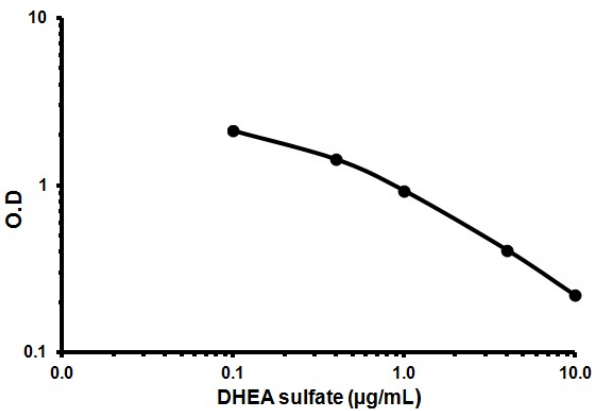
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in $\mu\text{g/mL}$.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (µg/mL)	O.D
0.0	2.84
0.1	2.12
0.4	1.43
1.0	0.93
4.0	0.41
10.0	0.22

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

Human serum and plasma DHEA sulfate reference values:

	Female µg/mL	Male µg/mL
Newborns	0.9 – 1.8	0.9 – 1.8
Before puberty	0.25 – 1.0	0.25 – 1.0
Adults	0.9 – 3.6	0.9 – 3.6
After menopause	< 0.25 – 1.0	
Pregnancy	0.25 – 1.8	

SENSITIVITY –

The lowest detectable concentration of DHEA-S that can be distinguished from Standard 0 is 0.04 µg/mL at the 95% confidence limit.

PRECISION –

	Intra-Assay	Inter-Assay
n=	64	48
%CV	≤ 7.9	≤ 10.4

RECOVERY –

The recovery of 0.6 - 1.25 - 2.5 and 5 µg/ml of DHEA-S added to sample gave an average value (±SD) of 102.87% ± 8.63 % with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 and 8 times gave an average value (±SD) of 100.15% ± 9.02 %.

16. ASSAY SPECIFICITY

16.1 The cross reaction of the antibody calculated at 50 % is:

DHEA sulfate	100 %
DHEA	100 %
Androstendione	59 %
Androsterone	16 %
DHT	1 %
Testosterone	0.63 %
Estrone	0.3 %
Progesterone	0.27 %
Pregnenolone	0.18 %
17-OH Progesterone	0.15 %
11-deoxycortisol	0.08 %
Cortisone	0.013%
Cortisol	< 0.01%
17 beta- Estradiol	< 0.01%
Corticosterone	< 0.01%
17a-Estradiol	< 0.01%
Cholesterol	< 0.001%
Estriol	< 0.001%
Estradiol Sulphate	< 0.001%
Aldosterone	< 0.001%
Estradiol-3-Sulphate-17-Glucuronide	< 0.001%

16.2 Interfering Substances

Interference by Bilirubin, Hemoglobin and Triglycerides has been investigated and no interference has been observed.

Substance	Assayed Conc.	Interference
Bilirubin	0.2 mg/ml	No
Hemoglobin	2 mg/ml	No
Triglycerides	6 mg/ml	No

16.3 Interfering Substances

Interference in plasma and SST (serum separation tube) samples has been evaluated. Serum obtained from the same patient has been used as reference. No interference has been observed.

Sample	Interference
SST (serum separation tube)	No
EDTA plasma	No
Lithium heparin plasma	No
Sodium-heparin plasma	No

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

RESOURCES

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

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