

SALIVARY DHEA-S ENZYME IMMUNOASSAY KIT

Catalog No. 1-1252, (Single) 96-Well Kit,
1-1252-5, (5-Pack) 480 Wells

For Research Use Only

Intended Use

The Salimetrics™ DHEA-S kit is a competitive immunoassay specifically designed for the quantitative measurement of salivary DHEA-S. It is not intended nor validated for diagnostic use. It is intended only for research use in humans and some animals. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Dehydroepiandrosterone-sulfate (DHEA-S) is a steroid hormone produced primarily in the adrenal cortex. DHEA-S is the most abundant steroid hormone in humans, with circulating concentrations approximately 250 and 500 times higher than those of its unsulfated analog, DHEA, in women and men, respectively.(1) DHEA-S serves as a precursor molecule that is circulated to various target tissues in the body. In those locations the sulfate is removed to yield DHEA, and the DHEA is then further metabolized into various estrogenic and androgenic compounds.(2) DHEA-S is not bound by sex hormone binding globulin (SHBG) in the blood stream and is readily available for conversion to other compounds. Unlike DHEA, DHEA-S does not normally exhibit any diurnal pattern of secretion.(1)

Levels of DHEA-S peak around the age of 20 to 30, and then decline to only 20-30% of peak levels by the age of 70 to 80.(1) Critical illness and emotional or physical stress can also cause DHEA-S levels to decline. Lowered DHEA-S levels have been linked with a variety of medical conditions, including rheumatic diseases,(3) cardiovascular disease,(4) immune system disorders,(5) and osteoporosis.(6) Elevated levels of DHEA-S have been tied to obesity and type II diabetes,(7) and to female hirsutism. (8)

DHEA-S and DHEA are also synthesized directly in the central nervous system, where they appear to help protect nervous tissue against harmful agents. (9,10) Studies have begun to explore possible relationships between DHEA-S levels and changes in neurological function, including sense of well-being, cognition, depression, and various other psychiatric disorders.(1,11)

DHEA-S is not lipid soluble, and it cannot enter saliva by passive diffusion through cell membranes like most of the other steroid hormones. Instead, it enters saliva only by squeezing through the tight junctions between cells in the saliva glands, and it is too large to do this readily. It is therefore present in relatively small amounts. Binding proteins or enzymes in saliva that would affect the measurement of free DHEA-S appear largely to be absent.(12)

DHEA-S levels measured in whole saliva may be inaccurate if contamination by plasma exudates from the gums or from small injuries in the mouth is present. (12) Subjects should be carefully screened for periodontal disease and advised about proper collection procedures. Saliva may also be screened for blood contamination using the Salimetrics Blood Contamination EIA Kit (Cat. No. 1-1302).

DHEA-S concentrations in saliva decrease markedly as flow rates increase. (12)

This kit is designed to measure DHEA-S levels in saliva. The standard is in a saliva-like matrix. In addition, a built-in pH indicator warns the user of acidic or basic samples.

Test Principle

A microtitre plate is coated with rabbit antibodies to DHEA-S. DHEA-S in standards and unknowns competes with DHEA-S linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound DHEA-S peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 3-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of DHEA-S peroxidase detected is inversely proportional to the amount of DHEA-S present. (13)

Precautions

1. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.
2. Liquid stop is a 3-molar solution of sulfuric acid. This solution is caustic; use with care.
3. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
4. Do not mix components from different lots of kits.
5. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
6. See 'Material Safety Data' at the end of procedure.
7. We recommend that samples be screened for possible blood contamination (14,15) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Cat. No. 1-1302). Do not use dipsticks, which result in false positive values due to salivary enzymes.
8. Routine calibration of pipettes is critical for the best possible assay performance.
9. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
10. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
11. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
12. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
13. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date. Use reconstituted standards and controls within 6 months.

Reagents and Reagent Preparation

1. **Anti-DHEA-S Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with rabbit anti-DHEA-S antibodies in a resealable foil pouch.
2. **DHEA-S Standard:** Lyophilized DHEA-S at a concentration of 15,300 pg/mL. Reconstitute with 1.5 mL of deionized water. (We recommend sterile water if you plan to store at 2 - 8°C.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. Use reconstituted standard within 6 months.
3. **DHEA-S Controls:** Two lyophilized controls representing high and low levels of DHEA-S in a saliva-like matrix with a non-mercury preservative. Reconstitute each vial with 1.0 mL of deionized water. (We recommend sterile water if you plan to store at 2 - 8°C.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. Use reconstituted controls within 6 months.

4. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H₂O). (*Note: If precipitate has formed in the concentrated wash buffer, heat to 40°C for 15 minutes to dissolve crystals. Cool to room temperature before use in assay.*)
5. **DHEA-S Assay Diluent:** 60 mL of a phosphate buffered solution containing a non-mercury preservative.
6. **Enzyme Conjugate:** 100 µL of a solution of DHEA-S labeled with horseradish peroxidase. Dilute prior to use with DHEA-S assay diluent.
7. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 12.5 mL of a 3-molar solution of sulfuric acid.
9. **Non-specific Binding Wells (NSB):** These wells do not contain anti-DHEA-S antibody. In order to support multiple use a strip of NSB wells is included in the foil pouch. These wells may be broken off and inserted as blanks (optional) where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 80 µL, 100 µL, 150 µL and 300 µL
- Precision multichannel pipette to deliver 50 µL, 150 µL, and 200 µL
- Vortex
- Plate rotator with 0.08-0.17 inch orbit
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One 18 mL disposable tube
- Small disposable tubes for dilution of standard, controls, and samples
- Pipette tips
- Serological pipette to deliver 18 mL

Specimen Collection

Due to the influence of saliva flow rates on DHEA-S levels, Salimetrics advises measuring the amount of time needed to collect the desired volume, then using this information to determine the flow rate. The measured concentration of DHEA-S (pg/mL) should then be multiplied by the flow rate (mL/min) to express the results as product measured per unit of time (pg/min).

Corrected DHEA-S (pg/min) = DHEA-S (pg/mL) x (Volume (mL) / Time (min))

Whole saliva may be collected by tilting the head forward, allowing the saliva to pool on the floor of the mouth, and then passing the saliva through a short straw into a polypropylene vial. Collection protocols are available on request. **Do not use Salivettes, the Salimetrics Oral Swab (SOS), Sorbettes, cotton, or polyester materials to collect samples.** Salimetrics internal data indicates that false readings will result. **Do not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected. Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Record the time and date of specimen collection. After collection it is important to keep samples cold, to minimize bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20°C within 4 hours of collection. (Samples may be stored at -20°C or lower for long term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. It is important to avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. **Note:** *It is important to keep the foil pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.*

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	15300 Std	15300 Std	C-L	C-L								
B	5100 Std	5100 Std	Unk 1	Unk 1								
C	1700 Std	1700 Std	Unk 2	Unk 2								
D	566.7 Std	566.7 Std	Unk 3	Unk 3								
E	188.9 Std	188.9 Std	Unk 4	Unk 4								
F	Zero	Zero	Unk 5	Unk 5								
G	NSB	NSB	Unk 6	Unk 6								
H	C-H	C-H	Unk 7	Unk 7								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder. Break off the bottom wells in each strip. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Cautions: 1. *Extra NSB wells should not be used for determination of standards, controls, or unknowns.*
2. *Do not insert wells from one plate into a different plate.*

Step 3:

- Label four microcentrifuge tubes or other small tubes 2 through 5.
- Pipette 300 µL of DHEA-S assay diluent into tubes 2 through 5. Serially dilute the standard 3X by adding 150 µL of the 15,300 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 150 µL from tube 2 and transfer to tube 3. Mix well. Continue for tubes 4 and 5. The final concentrations of standards for tubes 1 through 5, respectively, are 15,300 pg/mL, 5,100 pg/mL, 1,700 pg/mL, 566.7 pg/mL, and 188.9 pg/mL. Standard concentrations in nmol/L are 41.52, 13.84, 4.61, 1.54, and 0.51, respectively.
- Pipette 18 mL of DHEA-S assay diluent into a disposable tube. (Scale down proportionally if not using the full plate.) Set aside for Step 5.

Step 4:

- Pipette 100 µL of standards, controls, and unknown samples into appropriate wells. Standards, controls, and unknown samples should be assayed in duplicate.
- Pipette 100 µL of DHEA-S assay diluent into the duplicate zero and NSB wells.

Step 5: Dilute the enzyme conjugate 1:225 by adding 80 µL of the conjugate to the 18 mL of DHEA-S assay diluent prepared in Step 3. (Scale down proportionally if not using the full plate.) Immediately mix the diluted conjugate solution and add 150 µL to each well using a multichannel pipette.

Step 6: Cover plate with adhesive cover provided. Incubate at room temperature for 1 hour mixing constantly at 500 rpm.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then decanting the liquid into a sink. After each wash, blot plate on paper towels before turning upright. *If using a plate washer, blotting is still recommended after the last wash.*

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow. **Caution:** Do not mix at speeds over 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 630 is desirable.)

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.
5. If a dilution of the sample is used, multiply the results by the dilution factor.

Quality Control

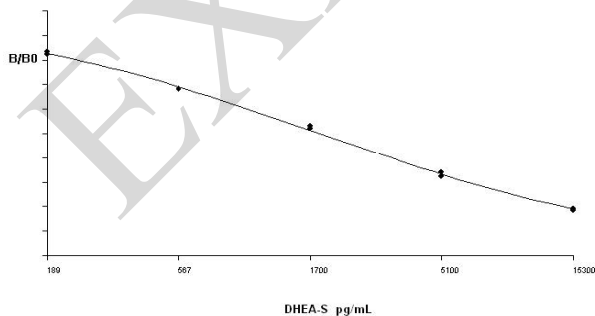
The Salimetrics' high and low salivary DHEA-S controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Typical Results

The following results are shown for illustration only and *should not* be used to calculate results from another assay.

Well	Standard	Average OD	B	B/Bo	DHEA-S (pg/mL)
A1,A2	S1	0.178	0.172	0.182	15300
B1,B2	S2	0.316	0.310	0.328	5100
C1,C2	S3	0.500	0.494	0.522	1700
D1,D2	S4	0.649	0.643	0.680	566.7
E1,E2	S5	0.788	0.782	0.827	188.9
F1,F2	Bo	0.952	0.946		NA
G1,G2	NSB	0.006			NA

Example: DHEA-S 4-Parameter Sigmoid Minus Curve Fit



Material Safety Data*

Hazardous Ingredients

Liquid stop solution is caustic; use with care. We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics shall not be liable for accidents or damage resulting from contact with reagents.

Performance Characteristics

A. Precision:

1. The intra-assay precision was determined from the mean of 18-24 replicates each.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	24	9635	548	5.7
Low	18	526	46	8.8

2. The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	12	9368	690	7.4
Low	12	519	40	7.7

B. Linearity of Dilution:

Three saliva samples were diluted with DHEA-S assay diluent and assayed.

Sample	Dilution Factor	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I			7650	
	1:2	3825	3478	90.9
	1:4	1913	1799	94.1
	1:8	956	769	80.5
	1:16	478	460	96.1
II			10287	
	1:2	5143	5861	114
	1:4	2572	2712	105.5
	1:8	1286	1437	111.8
	1:16	643	740	115.1
III			7652	
	1:2	3826	3157	82.5
	1:4	1913	1913	100
	1:8	956	876	91.6
	1:16	478	460	96.2

C. Recovery:

Two saliva samples were spiked with 3 different levels of DHEA-S and assayed.

Sample	Endogenous (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I	2055	9600	11491	11133	96.9
	2055	1530	3380	3701	109.5
	2055	170	2020	2299	113.8
II	2272	9600	11690	12686	108.5
	1733	1530	3089	3034	98.2
	1733	170	1729	1876	108.5

D. Sensitivity:

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 10 sets of duplicates at the 0 pg/mL level. The minimal concentration of DHEA-S that can be distinguished from 0 is < 43 pg/mL.

E. Specificity of Antiserum

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in Salivary DHEA-S EIA
Estradiol	10	ND
Estriol	1000	ND
Progesterone	1000	ND
17 α -Hydroxyprogesterone	1000	ND
Testosterone	1000	ND
Cortisol	1000	ND
DHEA	1000	ND
Androstenedione	1000	0.0844
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Dexamethasone	1000	ND
Triamcinolone	1000	ND
Corticosterone	1000	ND
Prednisolone	1000	ND
Prednisone	1000	ND
Transferrin	1000	ND
DHT	1000	ND
Dianabol	1000	ND
19-Nortestosterone	1000	ND
11-Hydroxytestosterone	1000	ND
Estrone	1000	ND
Transandrosterone	1000	0.0268

ND = None detected (<0.004)

*Salivary DHEA-S Expected Ranges:

Group	N	Mean (pg/min)	Standard Deviation (pg/min)
Male	19	2721	3082
Female	48	630	515

*Values adjusted for flow rate.

*To be used as a guide only. Each laboratory should establish its own range.

Citations

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Seller's Limited Warranty

“Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties”