SALIVARY DHEA-S

ENZYME IMMUNOASSAY KIT

For Research Use Only
Not for use in Diagnostic Procedures

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

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Intended Use

The Salimetrics® DHEA-S Enzyme Immunoassay Kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary DHEA-S. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Salimetrics has not validated this kit for serum or plasma samples.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

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.

DHEA-S and DHEA are also synthesized directly in the central nervous system, where they appear to help protect nervous tissue against harmful agents (3,4). 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 (1,5).

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 (6).

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 (6). 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 (Item. No. 1-1302).

DHEA-S concentrations in saliva decrease markedly as flow rates increase (6).

Test Principle

This is a competitive immunoassay kit. DHEA-S in standards and samples compete with DHEA-S conjugated to horseradish peroxidase for the antibody binding sites on a microtitre plate. After incubation, unbound components are washed away. Bound DHEA-S Enzyme Conjugate is measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The amount of DHEA-S Enzyme Conjugate detected is inversely proportional to the amount of DHEA-S present in the sample (7).

Safety Precautions

Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

Liquid Stop Solution is caustic; use with care. We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory practices when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using appropriate 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 call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

General Kit Use Advice

- This kit uses break-apart microtitre strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the foil pouch with desiccant and used in the frame provided.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volumes of wash buffer and enzyme conjugate prepared for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
- Do not mix components from different lots of kits.
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When using a multichannel pipette to add reagents, always follow the same sequence when adding all reagents so that the incubation time is the same for all wells.
- When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.
- 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 may affect OD values.
- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.
- When mixing plates during assay procedures, avoid speeds that spill the contents of the wells.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date.

Specimen Collection

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.

Collect whole saliva by unstimulated passive drool. Donors may tilt the head forward, allowing the saliva to pool on the floor of the mouth, then pass the saliva through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com or upon request.

Samples visibly contaminated with blood should be recollected. Samples may be screened for possible blood contamination (8,9) using our Blood Contamination EIA Kit (Item Nos. 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.

It is important to record the time and date of specimen collection.

Note: 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))

Sample Handling and Preparation

After collection, it is important to keep samples cold to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC for up to 6 months.) For long term storage, refer to the Salimetrics Collection and Handling Advice Booklet.

Do not add sodium azide to saliva samples as a preservative, as it may cause interference in the immunoassay.

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding and affect results. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Re-freeze saliva samples as soon as possible after adding to the assay plate. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

Materials Supplied with Single Kit

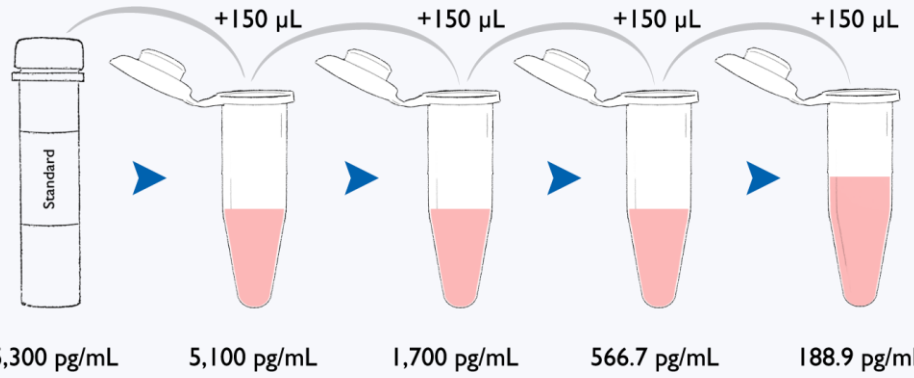
	Item	Quantity/Size
1	**Microtitre Plate** Coated with polyclonal anti-DHEA-S antibodies.	1/96 well
2	**DHEA-S Standard** 15300 pg/mL, in a saliva-like matrix. Serially dilute before use according to Reagent Preparation. Contains: DHEA-S, buffer, preservative.	1 vial
3	**DHEA-S Controls** High, Low, in a saliva-like matrix. Ready to use. Contain: DHEA-S, buffer, preservative.	2 vials
4	**DHEA-S Enzyme Conjugate** Concentrate. Dilute before use with DHEA-S Assay Diluent. (See step 5 of Procedure.) Contains: DHEA-S conjugated to HRP, preservative.	1 vial / 100 µL
5	**DHEA-S Assay Diluent** Contains: phosphate buffer, preservative.	1 bottle / 60 mL
6	**Wash Buffer Concentrate (10X)** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle / 100 mL
7	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle / 25 mL
8	**Stop Solution**	1 bottle / 12.5 mL
9	**Non-Specific Binding (NSB) Wells** Do not contain anti-DHEA-S antibody. Break off and insert as blanks (optional) where needed.	1 strip
10	**Adhesive Plate Covers**	2

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 capable of 500 rpm
- Plate reader with 450 nm and 620 to 630 reference filters
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One disposable polypropylene tube to hold at least 18 mL
- Four small disposable polypropylene tubes for dilution of standard
- Pipette tips
- Serological pipette to deliver up to 18 mL
- Centrifuge capable of 1500 x g

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 18 mL of DHEA-S Assay Diluent used in Step 5 (conjugate dilution) to come to room temperature.
- Bring microtitre plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may negatively influence the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized water). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay.)
- Prepare serial dilutions of the DHEA-S Standard as follows:
    - Label four polypropylene 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 to tube 3. Mix well.
    - Continue for tubes 4, and 5.
    - The final concentrations of standards for tubes 1 through 5 are, respectively, 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.



Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Standards, controls, and saliva samples should be assayed in duplicate.)

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

*NSB = Non-specific binding wells. These may serve as blanks. Use is optional.

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 G-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving G-1, 2 blank. Break off 2 NSB wells from the strip of NSB wells included in the foil pouch. Place in G-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the 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: Pipette 18 mL of DHEA-S Assay Diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 100 µL of standards, controls, and saliva samples into appropriate wells.
- Pipette 100 µL of DHEA-S Assay Diluent into 2 wells to serve as the zero.
- Pipette 100 µL of DHEA-S Assay Diluent into each NSB well.

Step 5: Dilute the enzyme conjugate 1:225 by adding 80 µL of the conjugate to the 18 mL tube of DHEA-S Assay Diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and add 150 µL to each well using a multichannel pipette.

Step 6: Place adhesive cover provided over plate. Mix plate on a plate rotator ***continuously*** at 500 rpm for 1 hour at room temperature.

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 discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted 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 Substrate Solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark (covered) 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. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

  Caution: Spillage may occur if mixing speed exceeds 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. (For best results, a secondary filter correction at 620 to 630 nm is recommended.)

Quality Control

The Salimetrics' High and Low 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.

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 OD of the zero, standards, controls, and saliva samples.
3. Calculate the percent bound (B/Bo) for each standard, control, and saliva sample by dividing the OD of each well (B) by the average OD for the zero (Bo). (The zero is not a point on the standard curve.)
4. Determine the concentrations of the controls and saliva samples by interpolation using data reduction software. We recommend using a 4-parameter non-linear regression curve fit.
5. Samples with DHEA-S values greater than 15,300 pg/mL should be diluted with DHEA-S Assay Diluent and rerun for accurate results. If a dilution of the sample is used, multiply the assay results by the dilution factor.

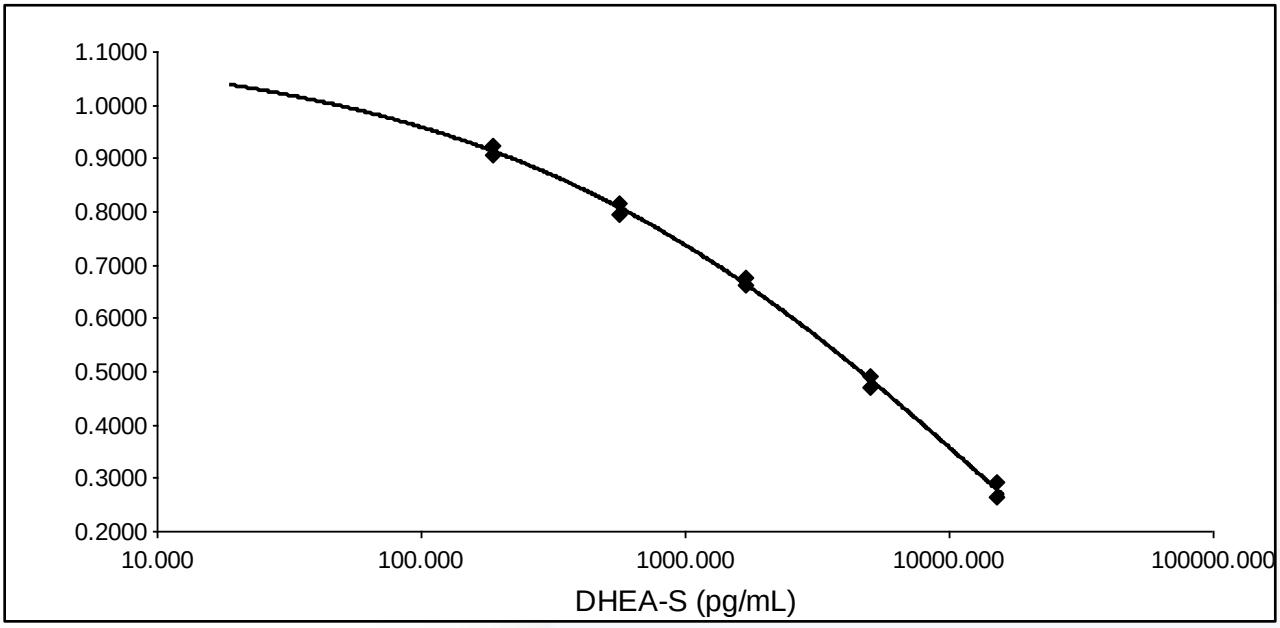
A new Standard Curve must be run with each full or partial plate.

Typical Results

The results shown below are 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	NA
G1,G2	NSB	0.006	NA	NA	NA

Example: DHEA-S 4-Parameter Curve Fit



Limitations

- Samples with DHEA-S values greater than 15,300 pg/mL should be diluted with DHEA-S Assay Diluent and rerun for accurate results. To obtain the final DHEA-S concentration, multiply the concentration of the diluted sample by the dilution factor.
- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.
- Samples collected with sodium azide are unsuitable for this assay.
- Any quantitative results indicating abnormal DHEA-S levels should be followed by additional testing and evaluation.

Salivary DHEA-S Example 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.

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Salivary DHEA-S EIA Kit Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 20 replicates each.

Saliva Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
1	20	9132.11	527.29	5%
2	20	7880.82	548.34	5%
3	20	5101.57	250.84	4%
4	20	4636.08	337.94	4%
5	20	1422.37	161.25	4%

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Saliva Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
1	20	8863.36	1045.36	12%
2	20	7470.01	625.24	8%
3	20	5983.23	457.55	8%
4	20	5133.09	371.38	7%
5	20	1382.87	172.96	13%

Recovery

Three saliva samples containing levels of an endogenous DHEA-S were spiked with known quantities of DHEA-S and assayed.

Saliva Sample	Endogenous (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1	4006.27	3473.88	7480.15	8172.15	109%
		814.24	4820.51	4571.77	95%
		93.01	4099.28	4089.28	100%
2	3600.02	3473.88	7073.90	7408.36	105%
		814.24	4414.26	4289.01	97%
		93.01	3693.03	3996.91	100%
3	3753.56	814.24	4567.80	4674.77	102%
		93.01	3846.57	3996.91	104%

Sensitivity

Analytical Sensitivity

The lower limit of detection (LLOD) 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 95.14 pg/mL.

Functional Sensitivity

The functional sensitivity was determined by assaying 20 saliva samples at a concentration level resulting in a CV of </= 20%. The functional sensitivity of the salivary DHEA-S ELISA is 239.67 pg/mL.

Sample Dilution Recovery

Three samples were serially diluted with DHEA-S Assay Diluent and assayed.

Saliva Sample	Dilution Factor	Expected (pg/ml)	Observed (pg/mL)	Recovery (%)
1			10494.69	
	1:2	5247.35	5337.24	102%
	1:4	2623.67	2414.82	92%
	1:8	1311.84	1231.97	94%
2			8775.60	
	1:2	4387.80	4274.23	97%
	1:4	2193.9	2020.61	92%
	1:8	1096.95	1055.57	96%
3			6011.37	
	1:2	3005.69	2846.83	95%
	1:4	1502.84	1442.89	96%
	1:8	751.42	768.09	102%

Linearity of Assay

Two saliva samples were diluted with each other proportionately and assayed.

Sample ID	Percentage of Sample		Observed (pg/ml)	Expected (pg/mL)	Recovery (%)
	High (L1)	Low (L11)			
L1 (High)	100%	0%	5567.59	5567.59	100%
L2	90%	10%	5940.41	5968.77	100%
L3	80%	20%	6291.01	6369.95	99%
L4	70%	30%	7021.93	6771.13	104%
L5	60%	40%	7313.16	7172.31	102%
L6	50%	50%	8695.41	7573.50	115%
L7	40%	60%	8743.13	7974.68	110%
L8	30%	70%	8864.11	8375.86	106%
L9	20%	80%	9089.86	8777.04	104%
L10	10%	90%	9211.98	9178.23	100%
L11 (Low)	0%	100%	9579.41	9579.41	100%
				Average=	104%

Antibody Specificity

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
Triamincinolone	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)

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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."

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