



# **SALIVARY DHEA**

## **ENZYME IMMUNOASSAY KIT**

For Research Use Only

Item No. 1-1202, (Single) 96-Well Kit;  
1-1202-5, (5-Pack) 480 Wells

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Must use IFU that is shipped with product.

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# Salivary DHEA EIA Kit

## Intended Use

The Salimetrics™ DHEA kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary dehydroepiandrosterone (DHEA). It is intended only for research use in humans and some animals.

***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 false 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

DHEA is a major secretory product of the adrenal glands; it is co-released along with cortisol in response to ACTH from the pituitary gland. (1) Circulating levels of DHEA decline rapidly after birth and remain low until about the age of 6-8, when they begin to rise. (2) This prepubertal onset of adrenal production, known as the adrenarche, involves enzymatic changes within the adrenal gland. (3) DHEA levels increase until the third decade of life, then they begin to decline again. Significant reductions occur after the age of 50. (4,5)

A major role of DHEA is to act as a circulating precursor for conversion to androgens and estrogens in tissues throughout the body, (5) and it has also been associated with immune function. (6) In addition, DHEA is produced directly in the nervous system, where it functions as a neuroactive and neuroprotective factor. (5,7,8)

In blood, only 1-10% of DHEA is in its unbound or biologically active form. The remaining DHEA is bound to serum proteins. Unbound DHEA enters the saliva via intracellular mechanisms; the majority of DHEA in saliva is non-protein bound. Salivary DHEA levels are unaffected by salivary flow rate or salivary enzymes. (9) DHEA exhibits a diurnal rhythm similar to cortisol, with highest levels in

the morning after awakening, followed by a decline throughout the afternoon and evening. (10)

This kit is designed to measure DHEA 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. DHEA in standards and unknowns competes with DHEA linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound DHEA 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 2-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of DHEA peroxidase detected is inversely proportional to the amount of DHEA present. (11)

## pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. DHEA values from samples with a  $\text{pH} \leq 4.0$  or  $\geq 9.0$  may be artificially inflated or lowered. (12)

## Storage

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

## Safety Precautions

- Liquid stop is a 2-molar solution of sulfuric acid. This solution is caustic; use with care.
- See "Material Safety Information" at the end of procedure.

## Materials Supplied with Single Kit

	Item	Quantity/Size
1	<b>Microtitre Plate</b> Coated with polyclonal anti-DHEA antibodies.	1/96-well
2	<b>DHEA Standard</b> 1000 pg/mL. Serially dilute before use according to Reagent Preparation. Contains: DHEA, buffer, preservative.	1 vial/1 mL
3	<b>DHEA Controls</b> High, Low. Ready to use. Contain: DHEA, buffer, preservative.	2 vials/500 $\mu$ L each
4	<b>Wash Buffer Concentrate (10x)</b> Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle/100 mL
5	<b>Assay Diluent</b> Contains: phosphate buffer, pH indicator, preservative.	1 bottle/60 mL
6	<b>DHEA Enzyme Conjugate</b> Concentrate. Dilute before use with assay diluent. (See step 5 of Procedure.) Contains: DHEA conjugated to HRP, preservative.	1 vial/50 $\mu$ L
7	<b>TMB Substrate Solution</b> Non-toxic, ready to use.	1 bottle/25 mL
8	<b>2 M Stop Solution</b> Contains: sulfuric acid.	1 bottle/12.5 mL
9	<b>Non-Specific Binding (NSB) Wells</b> Do not contain anti-DHEA antibody. Break off and insert as blanks (optional) where needed.	1 strip

## Materials Needed But Not Supplied

- Precision pipette to deliver 12  $\mu\text{L}$ , 50  $\mu\text{L}$ , 100  $\mu\text{L}$ , 150  $\mu\text{L}$  and 18 mL
- Precision multichannel pipette to deliver 50  $\mu\text{L}$ , 150  $\mu\text{L}$ , and 200  $\mu\text{L}$
- Vortex
- Plate rotator with 0.08-0.17 inch orbit (if unavailable, tap to mix)
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One 20 mL disposable tube
- Five small disposable tubes
- Pipette tips
- Serological pipette

## 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.

Due to the episodic secretion pattern of steroid hormones, we can expect reproducible and reliable results only in cases of multiple sampling. Therefore, we recommend taking a minimum of 3 samples over a 2-hour period. Equal volumes from each of the samples should be pooled to create one sample that physically averages the fluctuations over that time period. (13,14)

The preferred method for collecting whole saliva is by unstimulated passive drool. Donors may collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, then passing the saliva through a short straw into a polypropylene vial.

***Do not use Salivettes, the Salimetrics Oral Swab (SOS), the Salimetrics Children's Swab (SCS), the Salimetrics Infant's Swab (SIS), sorbettes, cotton, or polyester materials to collect samples. False readings will result. (15)***

Samples visibly contaminated with blood should be recollected. Samples collected from populations that have little or no dental care, or known oral health problems, may be screened for possible blood contamination (16,17) using our Blood Contamination EIA Kit (Item No. 1-1302/1-1312). 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 due to the diurnal variation in DHEA levels.

## **Sample Handling and Preparation**

After collection it is important to keep samples cold, in order to avoid 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.)

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

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. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Be careful not to disrupt the pellet, since particulate matter may interfere with antibody binding, leading to falsely elevated results.

Avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying.

## Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is necessary for the 18 mL of 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 have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting wash buffer concentrate 10-fold with room-temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H<sub>2</sub>O). ***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 standard as follows:
  - Label five microcentrifuge tubes or other small tubes 2 through 6.
  - Pipette 150 µL of assay diluent into tubes 2 through 6.
  - Serially dilute the standard 2.5X by adding 100 µL of the 1000 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, transfer 100 µL from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6 are, respectively, 1000 pg/mL, 400 pg/mL, 160 pg/mL, 64 pg/mL, 25.6 pg/mL, and 10.2 pg/mL. Standard concentrations in nmol/L are 3.47, 1.39, 0.55, 0.22, 0.09, and 0.03, respectively.

## 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.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volumes of wash buffer and 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.
- 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.
- 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 running multiple plates, or multiple sets of strips, a standard curve should 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 will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.
- Routine calibration of pipettes is critical for the best possible assay performance.

## Procedure

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000 Std	1000 Std	Control High	Control High								
B	400 Std	400 Std	Control Low	Control Low								
C	160 Std	160 Std	Unknown 1	Unknown 1								
D	64 Std	64 Std	Unknown 2	Unknown 2								
E	25.6 Std	25.6 Std	Unknown 3	Unknown 3								
F	10.2 Std	10.2 Std	Unknown 4	Unknown 4								
G	Zero	Zero	Unknown 5	Unknown 5								
H	NSB	NSB	Unknown 6	Unknown 6								

**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 and break off the bottom wells. 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 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 assay diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

#### Step 4:

- Pipette 50  $\mu\text{L}$  of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 50  $\mu\text{L}$  of assay diluent into 2 wells to serve as the zero.
- Pipette 50  $\mu\text{L}$  of assay diluent into each NSB well.

**Step 5:** Dilute the enzyme conjugate 1:1500 by adding 12  $\mu\text{L}$  of the conjugate to the 18 mL of assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and add 150  $\mu\text{L}$  to each well using a multichannel pipette.

**Step 6:** Place adhesive cover (provided) over plate. Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for a total of 3 hours.

**Step 7:** Wash the plate 4 times with 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  $\mu\text{L}$  of wash buffer into each well and then flipping the liquid into 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, just before the addition of the TMB.

**Step 8:** Add 200  $\mu\text{L}$  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  $\mu\text{L}$  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:** *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 490 to 630 nm is recommended.)

## Assay Summary

1. Bring all reagents to room temperature and mix before use.
2. Bring plate to room temperature and prepare for use with NSB wells. (Use of NSB wells is optional.)
3. Prepare tube with 18 mL of assay diluent for conjugate dilution, which will be made later.
4. Prepare 1X wash buffer.
5. Serially dilute DHEA standard.
6. Pipette 50  $\mu$ L of standards, controls, and unknowns into appropriate wells.
7. Pipette 50  $\mu$ L of assay diluent into zero and NSB wells.
8. Make final 1:1500 dilution of conjugate (12  $\mu$ L into 18 mL assay diluent), mix, and immediately pipette 150  $\mu$ L into each well.
9. Place adhesive cover over plate, then mix for 5 minutes at 500 rpm. Incubate at room temperature for 3 hours.
10. Wash plate 4 times with 1X wash buffer. Blot.
11. Add 200  $\mu$ L TMB solution to each well.
12. Mix plate for 5 minutes at 500 rpm. Incubate in dark at room temperature for 25 additional minutes.
13. Add 50  $\mu$ L stop solution to each well. Mix for 3 minutes at 500 rpm.
14. Wipe plate bottom clean and read within 10 minutes of adding stop.

## 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 is used for the sample, multiply the assay results by the dilution factor. Samples with DHEA values greater than 1000 pg/mL should be diluted with assay diluent and rerun for accurate results.

***When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.***

## Quality Control

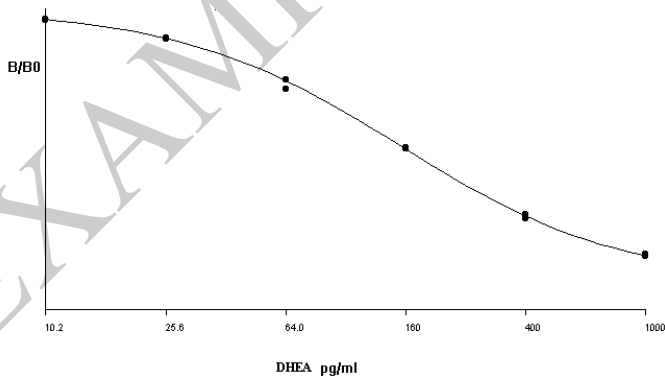
The Salimetrics' high and low salivary DHEA controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

## Typical Results

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

Well	Sample	Average OD	B	B/Bo	DHEA (pg/mL)
A1,A2	S1	0.344	0.295	0.234	1000
B1,B2	S2	0.513	0.464	0.368	400
C1,C2	S3	0.741	0.692	0.548	160
D1,D2	S4	0.958	0.909	0.720	64
E1,E2	S5	1.145	1.096	0.868	23.5
F1,F2	S6	1.23	1.181	0.936	10.2
G1,G2	Bo	1.311	1.262	NA	NA
H1,H2	NSB	0.049	NA	NA	NA

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



## **Material Safety Information\***

### ***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 be used only as a guide. Salimetrics shall not be liable for accidents or damage resulting from misuse of product.

## **Salivary DHEA EIA Kit Performance Characteristics**

### ***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 that can be distinguished from 0 is 5 pg/mL.

### ***Correlation with serum***

The correlation between serum and saliva DHEA was determined by assaying 39 matched samples using the Diagnostic Systems Laboratories serum DHEA radioimmunoassay and the Salimetrics Salivary DHEA EIA. The DHEA serum-saliva correlation, using a log 10 transformation for the total ( $n = 39$ ), combined males and females, is 0.857,  $p < 0.0001$ .

## Precision

1. The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
H	12	618.61	32.79	5.3
L	12	44.59	2.58	5.8

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 (%)
H	12	579.47	45.91	7.9
L	12	34.83	2.97	8.5

## Recovery

Six saliva samples containing different levels of an endogenous DHEA were spiked with known quantities of DHEA and assayed.

Sample	Endogenous (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1	84.45	50	134.45	136.21	98.7
2	66.84	400	466.84	511.34	109.5
3	317.92	50	367.92	334.18	90.8
4	317.92	500	817.92	919.4	112.4
5	41.84	16	57.84	64.49	111.5
6	185.04	16	201.04	181.30	90.2

## *Antibody Specificity*

<b>Compound</b>	<b>Spiked Concentration (ng/mL)</b>	<b>% Cross-reactivity in Salivary DHEA EIA</b>
DHEA-S	1000	0.063
Androstenedione	1000	0.0378
17- $\beta$ Estradiol	1	ND
Estriol	1000	ND
Estrone	1000	ND
Progesterone	1000	ND
17 $\alpha$ -Hydroxyprogesterone	1000	ND
Testosterone	1000	ND
Dihydroxytestosterone	1000	ND
Dianabol	1000	ND
11-Hydroxytestosterone	1000	ND
19-Nortestosterone	1000	ND
Cortisol	1000	ND
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Triamcinolone	1000	ND
Corticosterone	1000	ND
Transferrin	1000	ND

ND = None detected (<0.004)

## Linearity of Dilution

Two samples were serially diluted with assay diluent and assayed.

Sample	Dilution Factor	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1			334.66	
	1:2	167.33	157.33	94.0
	1:4	83.67	91.22	109.0
	1:8	41.83	49.44	118.2
	1:16	20.92	20.08	96.0
2			511.38	
	1:2	255.69	287.69	112.5
	1:4	127.84	140.12	109.6
	1:8	63.92	69.30	108.4
	1:16	31.96	34.25	107.2

Linearity is established from 500 to 20 pg/mL

## Method Comparison

The correlation between the Salimetrics EIA and a published serum RIA modified for use with saliva (15) was evaluated by assaying 40 common samples. The EIA-RIA results were highly correlated,  $r(38) = 0.881$ ,  $p < 0.001$ .

## Salivary DHEA Expected Ranges\*

Group	N	Range (pg/mL)	Mean (pg/mL)	Standard Deviation (pg/mL)
Females	19	15.9 - 291.1	165.6	71.6
Males	20	22.4 - 308.8	153.5	68.8

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

## References

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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.”**

EXAMPLE ONLY

Must use IFU that is shipped with product.

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Must use IFU that is shipped with product.

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Must use IFU that is shipped with product.