



DHEA-S Saliva Sensitive ELISA Assay Kit

Catalog Number: DHS32-K01

96 Wells

For Research Use Only

v. 2.0 (7/12/2020)

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I. Intended Use and Description

The Eagle Biosciences DHEA-S Saliva Sensitive ELISA Assay kit is designed and validated for the quantitative determination of Dehydroepiandrosterone Sulfate (DHEA-S) in human saliva by a microplate enzyme immunoassay (EIA).

II. Assay Background

The adrenal gland produces three main groups of hormones: glucocorticoids, mineral- corticoids and sex hormones. Clinical assessment of adrenal male sex hormones had depended in the past primarily upon measurement of the 24-hour urinary excretion of 17-ketosteroids. These urinary excretory products are derived predominantly from the weak androgen, dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S). DHEA is present in blood almost exclusively in its sulfated form DHEA-S. Preliminary studies to date have shown good correlation in most cases between urinary 17-ketosteroid values and serum DHEA-S (1). However, 17-ketosteroids do not always reflect production of adrenal or gonadal androgens. Under stressful situations, the metabolism of these steroids is affected, leading to excretion of non-17-ketosteroids (2). Also, certain drugs are known to produce the same effects (3). Under those conditions, the urinary 17- ketosteroid levels would underestimate the production rate of DHEA and DHEA-S by the gonads and adrenals. Lastly, but not least, the metabolites of

the potent 17 β -ol-androgen, testosterone and dihydrotestosterone, contribute very little to urinary 17-ketosteroids in women.

Measurement of plasma DHEA-S may be used instead of urinary 17-ketosteroids in evaluating conditions of adrenal androgen excess. Normally the ovary contributes less than 10% to plasma DHEA-S levels, whereas, the adrenal contributes over 90% (4,5). These proportions do not change significantly in cases of hirsutism due to ovarian androgen excess (6) in these cases, the ovary secretes excess of the potent 17 β -ol- androgens: Testosterone and dihydrotestosterone. Therefore, to evaluate the origin of androgen excess in cases of hirsutism, the measurement of plasma DHEA-S and the 17 β -ol-androgens will be of greater value than measurement of urinary 17-ketosteroids.

For the differential diagnosis of Cushing's syndrome, plasma DHEA-S and cortisol should be more informative than urinary 17-keto and 17-hydrosteroids. Elevation of plasma DHEA-S in this condition suggests a generalized state of adrenal overactivity. Carcinomas and adenomas of the adrenal have been associated with the highest plasma DHEA-S levels. Bilateral adrenal cortical hyperplasia frequently results in elevation of both DHEA-S and cortisol (7).

DHEA-S does not appear to be bound to proteins either in serum or saliva suggesting that DHEA-S exists in the free form (8,9).



In 1983 Vining, et. al (8,9) demonstrated that hormones can enter saliva by a variety of mechanisms. For the unconjugated or neutral steroids, the most common route is by rapid diffusion through the acinar cells and therefore their concentration suggests that they are independent of the rate of saliva flow. Conversely, for the conjugated or charged steroids like DHEA-S, the mode of entry is by diffusion between the tight junctions of the acinar cells and their salivary concentration suggest that they may be dependent on saliva flow.

The Eagle Biosciences DHEA-S ELISA Assay Kit uses a monospecific Anti-DHEA-S antibody **and does not measure DHEA** or other structurally related steroids.

III. Assay Principle

The Eagle Biosciences DHEA-Salivary Sensitive ELISA Assay Kit is based on the competition principal and microplate separation. An unknown amount of DHEA-S present in a saliva sample and a fixed amount of DHEA-S conjugated to horse radish peroxidase DHEA-S-HRP compete for binding sites with a rabbit polyclonal antiserum bound to GARGG (goat anti-rabbit gamma globulin) coated wells of a microplate. After incubation, unbound components are washed away. Enzyme substrate solution is then added and a blue color formed. This reaction is stopped with an acid solution to produce a yellow color. The optical density is then read at 450 nm. The amount of DHEA-S-HRP detected is inversely proportional to the amount of DHEA-S in a sample.

IV. Reagents Provided and Reagent Preparation

Store all other reagents at 2 to 8°C. Use only reagents supplied with this kit. Do not interchange reagents with different lot numbers. Expiration dates and lot numbers are printed on the labels.

1. GARGG Plate: One microplate (12x8 breakable strip wells) coated with goat anti-rabbit gamma globulin placed in a resealable foil bag with desiccant.
2. Salivary DHEA-S EIA Calibrators: 7 bottles. 5.0 mL of 0 calibrator, 1.0 mL of 0.1,0.5, 1, 3, 10 and 30 ng/mL.
3. Salivary DHEA-S EIA Control #1: 1 bottle, 1.0 mL. Concentration is on the label.
4. Salivary DHEA-S EIA Control #2: 1 bottle, 1.0 mL. Concentration is on the label.
5. Salivary DHEA-S EIA Antibody: 1 bottle, 6 mL of anti-DHEA-S. The solution is blue.
6. DHEA-S -Horse radish peroxidase (HRP) **concentrate (20X)**: 1 amber glassbottle 0.200 mL. The solution is light brown and light sensitive.



7. DHEA-S -Horse radish peroxidase (HRP) **conjugate buffer** 4 mL. The solution is yellow. **To be used for working reagent preparation only.**
8. DHEA-S -Horse radish peroxidase (HRP) **working reagent.**
Preparation:
Determine the amount of working DHEA-S-HRP needed and dilute 1:20 in conjugate buffer (number 7). For example, mix 0.125 mL of DHEA-S -HRP **concentrate** (number 6) + 2.375 mL (0.125 mL made up to a total volume of 2.5 mL) of DHEA-S-HRP conjugate buffer (number 7). This is sufficient for 100 wells. Discard the unused portion if not used within 36 hours of mixing.
9. Color Development Reagent EIA #1: 1 amber plastic bottle, 15 mL of tetramethylbenzidine plus hydrogen peroxide. Light sensitive.
10. Stopping Solution EIA #1: 1 bottle, 15 mL diluted acid solution.
11. **10X Wash Solution** EIA #1: 1 bottle, 50 mL. Prior to use determine the amount of **diluted** wash solution needed and dilute 1:10 with deionized water (example: 10 mL of 10X wash solution + 90 mL deionized water).

V. Materials Needed but not Supplied

- Device to dispense very accurately 50 μ L of saliva.
- Multichannel pipettors.
- Microplate or orbital shaker
- Vortex Mixer
- Microplate washer (not required, plates can be washed manually).
- Microplate reader capable of reading 450 nm with 4 parameter data reduction or comparable software.
- Plate sealer

VI. Precautionary Factors to Observe Before Saliva Sample Collection

- Do not add sodium azide to samples.
- Check for blood contamination (levels of DHEA-S in blood are approximately 1000X those in saliva) with commercially available blood detection kits.
- Avoid Collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol



VII. Sample Collection and Processing

Collection: This sample collection and processing procedure must be followed.

- Rinse mouth thoroughly with water 10 minutes prior to collection.
- Collect whole saliva by unstimulated passive drool by allowing saliva to drip off the lower lip into a graduated conical tipped plastic test tube or by allowing the saliva to accumulate in the floor of the mouth and spitting it into a test tube.
- Time and date specimen.
- Refrigerate sample within 30 minutes of collection and freeze at or below -20°C or lower within 4 hrs. For longer term storage store at -20C or lower. On day of assay thaw samples to facilitate precipitation of mucins. Centrifuge at 1500X g for ten minutes. Bring samples to room temperature and assay.

VIII. Assay Procedure Summary Flow Sheet

Calibrator DHEA-S Sample I.D. (ng/mL)	Calibrator Control Sample (µL)	HRP DHEA-S Working Reagent (µL)	Anti-DHEA-S (µL)		Diluted 10X Wash Solution (µL)			Color Developer Reagent (µL)		Stopping Solution (µL)				
0	50	25	50	Mix. Incubate for 2 hrs. at Room Temperature, shaking.	300		Wash 3X	125		125	Mix. Read at 450 nm			
0.1	50	25	50		300			125		125				
0.5	50	25	50		300			125		125				
1.0	50	25	50		300			125		125				
3	50	25	50		300			125		125				
10	50	25	50		300			125		125				
30	50	25	50		300			125		125				
Control #1	50	25	50		300			125		125				
Control #2	50	25	50		300			125		125				
Sample	50	25	50		300			125		125				



IX. Assay Procedure

1. To GARGG microplate dispense 50 μ L of ready-to-use Salivary DHEA-S EIA calibrators (0, 0.1, 0.5, 1.0, 3.0, 10.0 and 30 ng/mL), controls, and saliva samples.
2. Add 25 μ L of **DHEA-S-HRP Working Reagent** to all wells (see **Reagent Preparation Section-number 8**).
3. Add 50 μ L of **Anti-DHEA-S EIA antibody**.
4. Cover microplate with plastic sealer. Incubate by shaking on a microplate orbital shaker set at 500-900 rpm for **2 hrs.** at room temperature.
5. After incubation, decant the contents of the wells. Wash 3 times with 300 μ L of **Diluted Wash Solution** (see page 4, number 11). After the 3rd wash, invert GARGG microplate on an absorbent paper and tap dry.
6. Dispense 125 μ L of **Color Development Reagent** EIA #1 into each well. Shake briefly (manual). Cover microplate with plastic sealer. Incubate for 30 minutes at room temperature.
7. Dispense 125 μ L of **Stopping Solution** EIA #1 into each microtiter well of the GARGG plate. Shake briefly (manual). Color changes from blue to yellow.
8. Read at 450 nm on a microplate reader within 30 minutes.

Note: If samples exceed the highest calibrator, dilute with zero calibrator and make appropriate concentration correction.

X. Typical Results

Typical Calibration Curve			
Calibrators (ng/mL)	Mean Absorbance (450 nM)	%B/Bo	Value (ng/mL)
0	2.945	100.0	0.0
0.1	2.596	88.1	0.1
0.5	1.987	67.5	0.5
1.0	1.555	52.8	1.0
3.0	0.983	33.4	3.0
10	0.508	17.3	10
30	0.249	8.5	30
Control #1	1.600	54.3	0.974
Control #2	0.318	10.8	21.137
Sample #1	0.656	22.3	6.178
Sample #2	1.496	50.8	1.166
Sample #3	1.970	66.9	0.502



XI. Calculation

1. Most microplate reader manufacturers provide software for data reduction. Use a 4-parameter data reduction is suggested.
2. Alternately plot absorbance of calibrators versus concentration on semi-log graph paper and read the concentration of unknowns off the curve.

Analytical measuring range (AMR)	0.1 - 30 ng/mL
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XII. Expected Values

	Median	Range
Males:	3.0 ng/mL	0.09 - 7.1 ng/mL (n = 50)
Females:	2.1 ng/mL	0.09 - 4.9 ng/mL (n = 50)

It is suggested that each laboratory establish its own normal ranges

XIII. Performance Characteristics

A. Specificity of Antiserum:

C-19 Steroids:	% Cross-reactivity
DHEA-SO ₄	100.00
DHEA	0.0016
7 Keto-DHEA	0.0036
Testosterone	<0.001
5 DHT	<0.001
Androstenedione	0.0066
Androsterone	0.4080
C-21 Steroids:	% Cross-reactivity
Progesterone	<0.001
17 OH Progesterone	<0.001
Pregnenolone	<0.001
17 OH Pregnenolone	<0.001
17 OH Pregnenolone-SO ₄	0.0067
Desoxycorticosterone	<0.001
Corticosterone	<0.001
Cortisol	<0.001
11-Desoxycortisol	<0.001
Aldosterone	<0.001
C-18 Steroids:	% Cross-reactivity
Estradiol 17 β	<0.001
Estradiol 17	<0.001
Estrone	<0.001
Estriol	<0.001



Antiserum Cross Reactivities ratios are expressed as concentrations of unlabeled DHEA-S over the compound that displaces 50% of DHEA-S-enzyme conjugate from the antiserum.

B. Detection Limits

The analytical sensitivity of the Eagle Biosciences DHEA-S Salivary Sensitive ELISA Assay Kit was determined by subtracting 2 standard deviations from the mean of 20 replicate analysis of the zero calibrator. The lowest detectable level of salivary DHEA-S in an assay was found to be 0.068 ng/mL

C. Precision and Reproducibility

Intra-assay

The intra-assay precision was determined from the mean of 20 replicates of low and high samples.

Sample	N	Mean (ng/mL)	Standard Deviation (ng/mL)	%CV
Low	20	1.03	0.06	6.1
High	20	20.3	0.76	3.7

Inter-assay

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

Sample	N	Mean (ng/mL)	Standard Deviation (ng/mL)	%CV
Low	12	0.99	0.090	9.1
High	12	20.2	1.03	5.1



D. Linearity

Sample	Dilution Factor (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery %
1			7.03	
	1:2	3.51	3.80	108.2
	1:4	1.76	1.60	91.3
	1:8	0.88	0.84	96.1
	1:16	0.44	0.42	94.7
2			5.37	
	1:2	2.69	2.75	102.3
	1:4	1.34	1.21	90.2
	1:8	0.67	0.66	97.8
	1:16	0.34	0.32	95.9
3			4.21	
	1:2	2.10	2.29	108.7
	1:4	1.05	1.08	102.6
	1:8	0.53	0.48	90.3
	1:16	0.26	0.26	100.0
4			12.55	
	1:2	6.28	6.04	96.3
	1:4	3.14	2.86	91.3
	1:8	1.57	1.40	88.9
	1:16	0.78	0.71	90.6

E. Recovery

Six (6) saliva samples were spiked with various amounts of DHEA-S and the Percent recovery determined.

Sample	Endogenous (ng/mL)	Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
1	6.49	0.25	6.74	6.81	101.0
2	3.96	1.00	4.96	5.42	109.3
3	2.74	2.00	4.74	4.39	92.7
4	5.93	4.00	9.93	9.60	96.6
5	1.48	8.00	9.48	8.00	84.4
6	0.97	16.00	16.97	15.48	91.3



XIV. Limitations

- **The reagents are optimized to measure DHEA-S directly in saliva.**

XV. Precautions

- Only physician, clinical labs, research labs and hospital labs may acquire, possess and use the kit.
- Compare contents and packing list, if there is breakage or shortage, notify Pantex immediately.
- Do not pipet reagents by mouth.
- Do not smoke, eat or drink while performing assay.
- Wear disposable rubber gloves.
- Treat all saliva samples as potentially infectious.
- Do not mix reagent lot numbers or alter in any way the reagents in this kit. If this is done, Pantex will not be responsible for the performance of the assay.
- Avoid contact with Color Development Reagent (TMB). It contains solvents that can irritate skin and mucus membranes. If contact is made, wash thoroughly with water.
- Avoid contact with stopping solution. It contains acid. If contact is made, rinse thoroughly with water

XVI. References

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