COSMO BIO CO., LTD.
Inspiration for Life Science

YK290 DHEA (Saliva) EIA

Product Instructions

FOR LABORATORY USE ONLY

YANAIHARA INSTITUTE INC.

Contents

– Please read all the package insert carefully before beginning the assay –

YK290 DHEA (Saliva) EIA Kit

I. Introduction

DHEA (dehydroepiandrosterone) is a steroid hormone mainly secreted from the cortex of adrenal gland. DHEA serves as a precursor in testosterone and estrogen synthesis. The plasma levels of DHEA declines rapidly after birth and remain low level until about the age of 6-8; when they begin to rise on prepubertal, then a rapid rise from puberty and reach their maximum level at around age of twentys. It declines rapidly from fortys then; at age of fiftys, it is only about half the peak level; and by age of seventys, it declines to about 10% the peak level[1]. DHEA has about 10% of androgenic activity compared to that of testosterone, but the role of its physiological activities is not yet conclusively defined. It has been reported that DHEA may revolved in cholesterol and lipid metabolism, insulin sensitivity and secretion[2,3] and immune function[4,5,6]. Abnormal DHEA levels have been reported in schizophrenia and obesity[7,8]. In addition, DHEA is also produced directly in the nervous system, where it may function as a neuroactive and neuroprotective factors[9].

In blood, over than 90% of DHEA is bound to a plasma protein called corticosteroid-binding globulin (CBG) and plasma albumin, some parts of unbound free DHEA are secreted to saliva. Majority of DHEA in saliva remains unbound to protein. The levels of saliva DHEA are not affected with saliva secretion flow rate, and have good correlation between DHEA measurements in saliva and serum. Furthermore, saliva DHEA is relatively stable to the degradation by enzymes and freezing-thawing cycles. 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].

The newly developed DHEA (saliva) EIA kit by our laboratory provides a high sensitivity, quantitative tool for direct determination of DHEA in saliva without pre-treatment for sample. Furthermore, assays using this kit can be completed within a short period. The DHEA (saliva) EIA kit newly developed will be a quite useful tool for further development in DHEA research.

YK290 DHEA (Saliva) EIA Kit	Contents
▼ The kit assay range: 22.2-5400pg/mL.	1) Antibody Coated Plate
▼ The assay running time: 3h. + 0.5 h.	2) DHEA Standard
▼ Maximum measurable samples: 41 in duplicate	3) HRP-Labeled DHEA
▼ Test sample: saliva.	4) Buffer Solution
▼ The 96-well plate in the kit is consisted of 8-wells strips, so that the kit can be used dividedly in strips.	5) TMB Substrate
Intra-assay %CV: 1.7~3.6	6) Concentrated Wash Solution
Inter-assay %CV: 5.5~8.5	7) Reaction Stopping Solution
	8) Adhesive Foil
Store all the components in the kit at 2-8℃.	
The expiry date is stated on the package.	

II. Characteristics

This EIA kit is used for quantitative determination of DHEA in saliva. It has various advantages, such as no extraction procedure of samples, short assay time, practically no influences of other physiological active substances coexisting in samples assayed.

< Specificity **>**

The specificity of this EIA kit is shown on page 8.

< Assay Principle **>**

This EIA kit for determination of DHEA is based on a competitive enzyme immunoassay using combination of specific antibody to DHEA and DHEA-horseradish peroxidase (HRP) conjugate (HRP-labeled DHEA) system. The 96 wells plate is coated with DHEA specific antibody, to which DHEA standard or samples, HRP-labeled DHEA are added for competitive immunoreaction. After incubation and plate washing, HRP enzyme activity is determined by 3,3',5,5'-tetramethylbenzidine (TMB) and the concentration of DHEA is calculated.

III. Composition

	Component	Form	Quantity	Main Ingredient
1	Antibody Coated Plate	microtiter plate	1 plate (96 wells)	Anti-DHEA antibodies
2	DHEA Standard	lyophilized powder	1 vial (36.45ng)	Synthetic DHEA
3	HRP-Labeled DHEA	Liquid	1 vial (0.6mL)	HRP conjugated DHEA
4	Buffer Solution	liquid	1 bottle (30 mL)	Casine-containing citrate Na buffer
5	TMB Substrate	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
6	Concentrated Wash Solution	liquid	1 bottles (50 mL)	1% Tween20 concentrated saline
7	Reaction Stopping Solution	liquid	1 bottle (12 mL)	1M sulfuric acid
8	Adhesive Foil		2 sheets	

IV. Method

< Equipment required **>**

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system (optional)
3. Micropipettes for volumes between 50 μL –1000 μL
4. Multi-channel pipettes for 8 or 12 wells and the tips

5. Polypropylene tubes for preparation of standard solutions
6. A microplate shaker (210-220 rpm)
7. Graduated cylinder (1000 mL)
8. Distilled or deionized water

< Preparatory work **>**

1. Preparation of DHEA standard solution: Reconstitute lyophilized **DHEA Standard** with 450μL of **Buffer Solution** which affords 81000pg/mL standard solution. The reconstituted DHEA standard solution (40μL) is diluted with 560μL of **Buffer Solution** that yields 5400pg/mL standard solution. The standard solution (5400pg/mL) (200μL) is diluted with 400μL of **Buffer Solution** to yield 1800pg/mL of standard solution. Repeat the same dilution to make standard solution of 600, 200, 66.7, and 22.2pg/mL, respectively. **Buffer Solution** is used as 0pg/mL.
2. Preparation of HRP-labeled DHEA solution: Take 533μL of **HRP-Labeled DHEA** from the labeled vial to dilute with 16mL of **Buffer Solution** completely just before use.
3. Preparation of wash solution: Dilute **Concentrated Wash Solution** (50 mL) to 1000 mL with distilled or deionized water.
4. Other reagents are ready for use.

< Assay procedure **>**

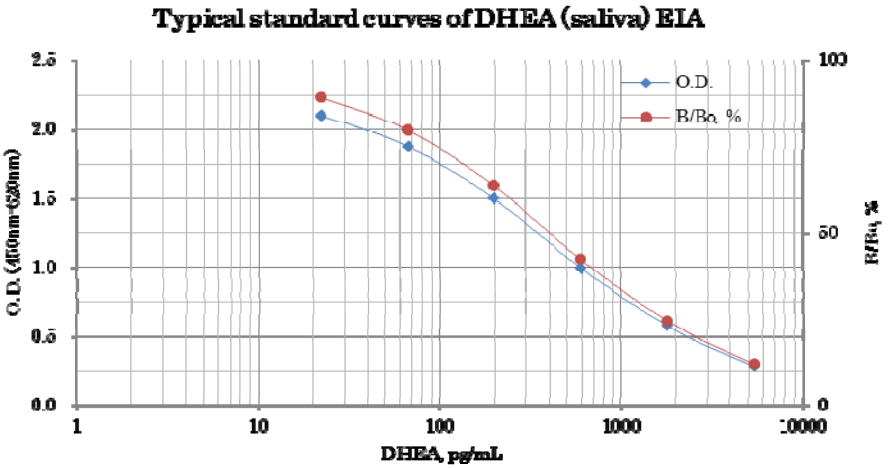
1. Before starting assay, bring all the reagents to room temperature (22-25°C).
2. Keep the desired number of strips in the plate holder and place the remaining strips back in the foil pouch.
3. Pipet 25 μL of DHEA standard solutions (0, 22.2, 66.7, 200, 600, 1800 and 5400pg/mL) or samples, after vortexed, into appropriate wells. Add 150μL of HRP-labeled DHEA solution into each well.
4. Cover the plate with adhesive foil and incubate it on a shaker at 210-220 rpm at room temperature for 180 minutes.
5. After incubation, take off the adhesive foil, aspirate or decant the solutions in the wells. Add 350μL of diluted wash solution to each well and keep it for about 30 seconds, and then aspirate or decant the wash solution in the wells. Repeat this wash process 6 times (total 7 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.
6. Add 100μL of **TMB Substrate** into each well.
7. Cover the plate with adhesive foil and incubate it on a shaker at 210-220rpm at room temperature for 30 minutes.
8. Add 100 μL of **Reaction Stopping Solution** into each well to stop color reaction.
9. Read the optical absorbance of the wells at 450nm (if possible, read 620nm for correction).
10. The assay fits best to a 4 (or 5)-parameter logistic equation, Y= (a-d)/(1+(x/c)^b)+d; here a,b,c,d represent constant parameter. Alternatively, calculate mean optical density values of wells containing standard solutions or their percent bound to maximum binding wells (0 pg/mL) and plot a standard curve on a semi-logarithmic graph paper (abscissa: concen-

trations of standard; ordinate: optical density or bound%). Use the average optical density or bound% of each sample to determine the corresponding value by simple interpolation from the standard curve. The results should be multiplied by the diluting factor to obtain the actually concentrations for undiluted unknown samples.

V. Notes

1. It is recommended that saliva samples should be collected by using special colleting tubes such as Saliva Collection Aid (Item No. 5016.02, Salimetrics LLC) or drool saliva directly into a polypropylene vial. If the sample is tested later, it should be frozen below −30°C. Avoid repeated freezing and thawing of samples.
2. On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 X g (@3000rpm) for 15 minutes. Transfer the supernatant of saliva carefully to another vial.
3. Avoid sample collection within 60 minutes after eating a meal or within 12 hours after consuming alcohol.
4. Avoid taking acidic or high sugar foods and caffeine drinks before collection.
5. To avoid the influences of food ingredients, rinse mouth thoroughly with water, then collected after an interval for at least 10 minutes before collection. Tooth brushing is not recommended.
6. Do not use samples contaminated with blood.
7. Do not use sodium azide as a preservative for collected samples.
8. DHEA standard solutions and HRP-labeled DHEA solution should be prepared immediately before use. If the kit used dividedly, dilute the propriety quantity of **HRP Labeled DHEA**. The rest of the reconstituted DHEA standard solution (81000pg/mL), **HRP-Labeled DHEA** and **TMB substrate** except wash solution and **Reaction Stopping Solution** should be stored at 4°C and used within 2 weeks. Diluted standard solutions and diluted HRP-labeled DHEA solution should not be reused for another assay.
9. Incomplete washing of the microplate will interfere with assay precision. If a microplate washer is not available, completely aspirate the solutions in the wells of assay plate to be removed or decant them by inverting the plate and tapping it onto absorbent tissue in each wash cycle. Ensure that there is no residual wash solution in the wells after final wash.
10. As pipetting operations may affect precision of the assay, pipet DHEA standard solutions or samples precisely into the wells of assay plate. In addition, use clean vials or vessels in assay and a new tip for each standard diluting process and for each sample or standard solution pipetting to avoid cross contamination.
11. Perform all the determination in duplicate.
12. To quantitate accurately, always run a standard curve for each assay.
13. Color reaction should be carried out under the light proof condition.
14. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
15. Protect the reagents from strong light (e.g. direct sunlight) during storage and assay.
16. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.
17. The recommended room temperature is 22-25℃

VI. Performance Characteristics



< Assay range **>** 22.2– 5400pg/mL

<Sensitivity**>**

Sensitivity can be calculated using the following formula under the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols [11].

$$\text{Sensitivity (pg/mL)} = \frac{2\times\text{SD of the Zero Standard}\times 22.2\text{pg/mL}}{(\text{Optical Density of 0pg/mL} - \text{Optical Density of 22.2pg/mL})}$$

< Precision and reproducibility **>**

Saliva sample	Intra-assay variation (mean±SD, n=10)		Inter-assay variation (mean±SD, n=9)	
	Measured (pg/mL)	%CV	Measured (pg/mL)	%CV
1	145±6	3.6	150±13	8.5
2	888±19	2.1	909±53	5.9
3	2802±49	1.7	2915±159	5.5

< Analytical recovery **>**

Saliva sample	DHEA added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
A	0	37.9		
	65	96.2	102.9	93.5
	196	193.7	233.9	82.8
	588	541.1	625.9	86.4

B	0	189.4		
	65	257.7	254.4	101.3
	196	402.7	385.4	104.5
	588	819.2	777.4	105.4
C	0	85.6		
	65	144.3	150.6	95.9
	196	252.5	281.6	89.7
	588	607.4	673.6	90.2
D	0	99.2		
	65	169.6	164.2	103.2
	196	260.2	295.2	88.1
	588	568.5	687.2	82.7
E	0	72.4		
	65	133.6	137.4	97.3
	196	245.4	268.4	91.4
	588	619.7	660.4	93.8
F	0	52.1		
	65	113.5	117.1	96.9
	196	213.4	248.1	86.0
	588	577.9	640.1	90.3

<Dilution test**>**

Saliva sample	Dilution ratio, 1X	Observed(pg/mL)	Expected(pg/mL)	% Of expected
No.1	1	46.7		
	2	19.8	23.4	85.0
	4	9.6	11.7	91.9
	8	-	-	-
No.2	1	213.9		
	2	103.3	107	96.6
	4	46.7	53.5	87.2
	8	25.6	26.7	95.8
No.3	1	95.4		
	2	45.3	47.7	95.1
	4	18.1	23.8	75.9
	8	-	-	-
No.4	1	119.8		
	2	50.6	59.9	84.4
	4	22.0	29.9	73.4
	8	-		-

No.5	1	80.5		
	2	37.8	40.2	93.9
	4	16.8	20.1	83.3
	8	-	-	-
No.6	1	62.2		
	2	26.7	31.1	85.8
	4	15.3	15.6	98.3
	8	-	-	-
No.7	1	108.7		
	2	46.9	54.4	86.4
	4	25.1	27.2	92.4
	8	9.8	13.6	72.2
No.8	1	71.5		
	2	30.3	35.8	84.7
	4	14.9	17.9	83.1
	8	7.6	8.9	85.0

<Cross reactivity**>**

Cross reactivities of the antibody used in the kit.

Name of substances	% Cross-reactivity
DHEA-S	0.067
Androstenedione	2.178
Aldosterone	ND
Cortisone	ND
Corticosterone	ND
Cortisol	ND
11-Deoxycortisol	ND
21-Deoxycortisol	ND
Danazol	0.006
Estriol	0.007
Estrone	0.030
17β-Estradiol	0.004
Progesterone	0.059
11α-Hydroxyprogesterone	ND
17α-Hydroxyprogesterone	0.019
Testosterone	2.118
Triamcinolone	ND

Cross-reactivities at B/Bo=50%; ND=None detected (<0.001%)

VII. Stability and Storage

< Storage **>** Store all the components in the kit at 2°C - 8°C.

< Shelf Life **>** The Kit is stable under the storage condition for 6 months from the date of manufacture.
The expiry date is stated on the label of package.

< Package **>** For 96 tests per one kit including standards.

VIII. References

1. J P Hinson and P W Raven: **DHEA deficiency syndrome: a new term for old age?** Journal of Endocrinology 163, 1–5, 1999
2. K Villareal DT, Holloszy JO: **Effect of DHEA on abdominal fat and insulin action in elderly women and men: a randomized controlled trial.** Jama 292:2243-8, 2004
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4. Casson PR et al : **Oral dehydroepiandrosterone in physiologic doses modulates immune function in postmenopausal women.** Am J Obstet Gynecol 169:1536-9, 1993
5. Rearte B et al: **Dehydroepiandrosterone and metyrapone partially restore the adaptive humoral and cellular immune response in endotoxin immunosuppressed mice.** Innate Immun 20(6):585-97, 2014
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8. Akyürek N et al: **Is there a relationship between cardiovascular risk factors and dehydroepiandrosterone sulfate levels in childhood obesity?** J Pediatr Endocrinol Metab 28(5-6):545-50, 2015
9. Stárka L et al: **Dehydroepiandrosterone: a neuroactive steroid.** J Steroid Biochem Mol Biol 145:254-60, 2015
10. Hucklebridge F et al: **The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to wakening.** Psychoneuendocrinology 30(1):51-7, 2005
11. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1 (1989), Vallanova, PA: NCCLS

IX. Appendix

The YK290 DHEA (Saliva) EIA kit has been compared to Salimetrics Salivary DHEA Enzyme Immunoassay Kit（Item No. 1-1202）. Twenty-five samples of saliva from normal volunteers were assayed and linear regression analysis of the results yielded as shown in the graph.

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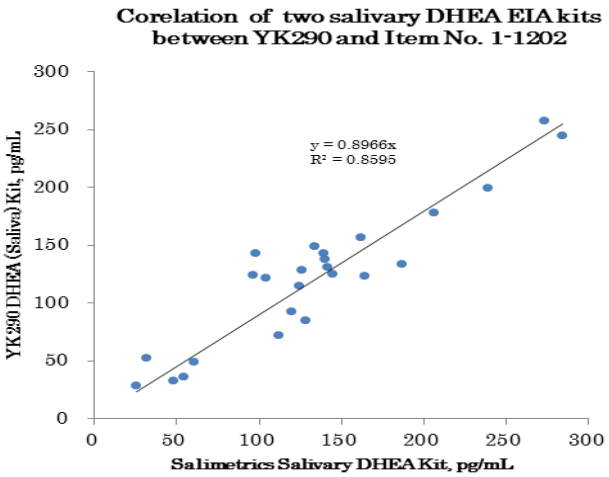

< Manufacturer >

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