

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone
	(CORTISOL SALIVA)		
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-3500

Cortisol Saliva

ELISA KIT Cat. #. 3500

For Quantitative Determination of Cortisol
In Human Serum

For In Vitro Research Use Only



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Cortisol Saliva ELISA Kit Cat. #. 3500

Kit Components	96 tests
Anti-Cortisol coated strip plate (96 wells), Cat. 3501	1 plate
Cortisol Saliva Std. A (0 ng/ml), 10 ml , Cat # 3502A	1 vial
Cortisol Saliva Std. B (1 ng/ml), 0.6 ml , Cat # 3502B	1 vial
Cortisol Saliva Std. C (3 ng/ml), 0.6 ml , Cat # 3502C	1 vial
Cortisol Saliva Std. D (10 ng/ml), 0.6 ml , Cat # 3502D	1 vial
Cortisol Saliva Std. E (30 ng/ml), 0.6 ml , Cat # 3502E	1 vial
Cortisol Saliva Std. F (100 ng/ml), 0.6 ml , Cat # 3502F	1 vial
Cortisol Saliva Control Serum, 0.6 ml; Cat # 3503 (refer to concn on the vial)	1 vial
Assay buffer, 15 ml; Cat # 3 5 0 4	1 bottle
Cortisol-HRP Conjugate (50X), 300 ul Cat # 3 5 0 5 , Dilute 1:50 in assay buffer before use,	1 bottle
TMB substrate, 16 ml, Cat # 3506	1 bottle
Stop solution (1N H2SO4), 6 ml; Cat. # T-30	1 bottle
Wash buffer (10X), 50 ml, Dilute 1:10, WB-10	1 bottle
Instruction Manual, M - 3 5 0 0	1

Introduction

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. It is increased under stress situations , physical exercise and external administration of ACTH. Measurement of cortisol levels in general can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia and carcinoma. Most circulating cortisol is bound to cortisol binding globulin or transcortin and albumin. The free cortisol, which is considered the active part of blood, is about 1-2%. In the absence of appreciable amounts of the cortisol binding proteins in saliva , salivary cortisol is considered to be free and shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

ADI's Cortisol Saliva ELISA is direct competitive ELISA kit for the measurement of Cortisol in saliva. It is not validated for other biological fluids.

Inter-assay precision: Three samples (mean concn 6.3, 23.7, and 51.8 ng/ml) were assayed ten times over a period of four weeks. SD_± were 0.63, 2.06, and 3.37 with CV% 9.8, 8.7, and 6.5 respectively.

3. LINEARITY

Three patient saliva samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

Sample	Observed	Expected	Recovery%
1	18.18	-	-
1:2	10.32	9.09	113.5
1:4	5.09	4.55	112.1
1:8	2.20	2.27	96.7
2	49.89	-	-
1:2	28.03	24.95	112.3
1:4	13.29	12.47	106.6
1:8	7.97	7.24	110.1
3	68.53	-	-
1:2	34.27	31.49	91.9
1:4	17.13	13.81	80.6
1:8	8.57	7.48	87.3

4. Recovery

Spiked samples were prepared by adding defined amounts of cortisol to three patient saliva samples (1:1).

Samples	Observed.	Expected	Recovery %
1 Unspiked	6.28	-	-
+ 1.0	4.14	3.64	113.7
+ 10	9.05	8.14	111.2
+ 100	61.85	53.14	116.4
2 Unspiked	8.03	-	-
+ 3.0	6.05	5.52	109.6
+ 30	20.64	19.02	108.5
+ 100	52.20	54.02	96.6
3 Unspiked	6.98	-	-
+ 3.0	5.38	4.99	107.8
+ 10	8.76	8.49	103.2
+ 30	19.00	18.49	102.8

5. SPECIFICITY AND CROSSREACTIVITY

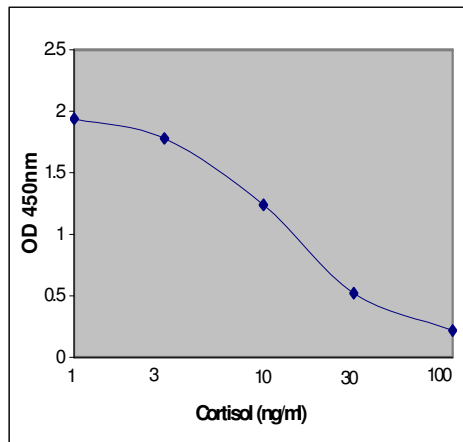
The specificity of CORTISOL SALIVA ELISA kit was determined by measuring interference from high concentrations of the following: Cortisol (100%), Prednisolone (13.6%), Corticosterone (7.6%), Deoxycorticosterone (7.2%), Progesterone (7.2%), Cortisone (6.2%), Deoxycortisol (5.6%), Pednisone (5.6%), Dexamethasone (1.6%). No cross reaction was detected with DHEAS and Tetrahydrocortisone. Please note that there is an observed cross-reactivity of 13.6% with prednisolone. Since prednisone is converted to prednisolone in vivo, caution must be exercised when assaying the cortisol levels of patients undergoing either therapy.

General References: Check JH (1995) Gynecol. Obst. Invest. 40, 139-140; Peters JR (1982) Clin. Endocrinol. 17, 583; Silver AC (1983) Clin. Endocrinol. 29, 1869; Brock P (1978) Clin. Chem. 24/9; 1595

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A ₄₅₀	Calculated Concn (ng/ml)
A1, A2	Std. A (0 ng/ml)	2.187	
B1, B2	Std. B (1 ng/ml)	1.940	
C1, C2	Std. C (3 ng/ml)	1.778	
D1, D2	Std. D (10 ng/ml)	1.238	
E1, E2	Std. E (30 ng/ml)	0.521	
F1, F2	Std. F (100 ng/ml)	0.219	
G1, G2	Sample 1	0.285	63 ng/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Cortisol Saliva ELISA kit is **1 ng/ml**.

2. PRECISION

Intra-assay precision: Three samples were assayed ten times each on the same calibrator Curve.

Sample	Mean (ng/ml)	SD	CV%
1	6.6	0.68	10.3
2	24.8	1.98	8.0
3	52.4	3.40	6.5

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. A set of standards is used to plot a standard curve from which the amount of cortisol in patient samples and controls can be directly read.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

The Alpha Diagnostic International CORTISOL SALIVA ELISA kit is intended for *in vitro* research use only. The stds./controls sera may contain human serum that has been shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND STORAGE

Approximately 1 ml of saliva is required per duplicate determination. Collect 4-5 ml of saliva into a clean glass tube (Salivette by Sarstedt may be used) without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed and centrifuged. The supernatants are to be collected and poured into freshly labelled tubes.

REAGENTS PREPARATION FOR THE ASSAY AND STORAGE

Cortisol-HRP Conjugate. Dilute 1:50 in assay buffer (prepare ml for a full 96 well plate. 240 µl in 12 ml of the assay buffer). Do not store diluted solution. Prepare in required amounts only. Store stock solution at 4°C.

Prepare 1X wash Buffer by diluting 10X stock in water (50 ml stock in 450 ml water) Store at 4°C and use at room temp.

Wash Buffer Concentrate

Dilute 1:10 in distilled water before use. Occasionally, some buffer components may crystallize that will dissolve at room temperature. Store stock solution at 4°C.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions. After opening the kit components, the shelf life is approx. 2 months.

TEST PROCEDURE (*ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE*). Dilute Wash buffer (1:0 with water) and CORTISOL SALIVA-HRP conjugate (1:50 in assay buffer).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate. Pipet **50 ul stds., controls, and samples** into appropriate wells.
2. Pipet **100 ul of Diluted CORTISOL-HRP conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **45 minutes** at room temperature (28-30 °C) **on a plate shaker** (approx 200 rpm).
3. Aspirate and **wash the wells 3 times with 300 ul of diluted wash buffer**. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **150 ul of HRP-substrate soln. (TMB)** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **15-20 minutes** at room temperature until dark blue color (A450≈2.0) develops in std A. The reaction can be stopped sooner or prolonged until desired color is obtained.
5. Stop the reaction by adding **50 ul of stopping solution to all wells**. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least one hr after stopping. If the A450 exceeds the upper limit of detection of the reader or if the 450nm filter is not available, Read at 405nm or 415nm (reading will be less but it will not affect the results).

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision.

Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than CORTISOL SALIVA should be first diluted with the zero std and then run with the standards and control) as described in the assay procedure. The results obtained should be multiplied by the appropriate first dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate CORTISOL SALIVA concentrations. Read off the CORTISOL SALIVA concentrations of the control and patient samples.

EXPECTED VALUES

It is expected that each lab determines its own normal range for the population it serves. The following values are given for reference purpose only.

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Random male and female samples were taken in the early morning and had an absolute range of: **5-21.6 ng/ml**