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 Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#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-1875

ALDOSTERONE

ELISA KIT Cat. No. 1875

For Quantitative Determination of Aldosterone In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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ALDOSTERONE ELISA KIT Cat. # 1875 (kit Contents)

Components	Cat. #
Rabbit Anti-Aldosterone IgG Coated Microwell strip **plate** (96 wells)	1 8 7 6
Aldosterone **Std. A,** 10 ml	1 8 7 7 A
Aldosterone **Std. B,** 0.5 ml	1 8 7 7 B
Aldosterone **Std. C,** (0.5 ml	1 8 7 7 C
Aldosterone **Std. D,** 0.5 ml	1 8 7 7 D
Aldosterone **Std. E,** 0.5 ml	1 8 7 7 E
Aldosterone **Std. F**, 0.5 ml	1 8 7 7 F
Note: See exact conc. On the vials	
Aldosterone **Low** & **High Controls** 0.5 ml (see vial for lot sp. concn) ;1 8 7 7 H C , 1 8 7 7 L C	
Aldosterone-**Avidin Conj**. (50X), 300 ul	1 8 7 8 B
Assay Buffer, ready to use, 15 ml	1 8 7 9
Wash Buffer Conc. (10X), 50 ml	W B 1 8 7 5
HRP substrate (**TMB**) Solution; 16 ml	T M 1 8 7 5
Stop solution, 6 ml	T - 3 0
Complete Instruction Manual	M 1 8 7 5

Intended use: ADI's Aldosterone ELISA is a rapid, specific and sensitive assay for the measurement of aldosterone in human urine or serum. For In Vitro Research Use Only (RUO)

Introduction- Aldosterone is a potent mineral corticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the re-absorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension. Measurement of aldosterone levels in serum in conjunction with plasma renin levels can be used to differentiate between primary and secondary aldosteronism.

Conditions	Serum Aldosterone	Plasma Renin
Primary Aldosteronism	Low	High
Secondary Aldosteronism	High	Low

The measurement of aldosterone in concert with selective suppression and stimulation tests can be used to further differentiate primary aldosteronism into two basic types:

- Primary aldosteronism caused by an adenoma of one or both adrenals.
- Primary aldosteronism caused by adrenal hyperplasia.

This differentiation is vital in the treatment and management of the disease. The adrenal adenomas respond well to surgery whereas hyperplastic disease of the adrenals is generally better managed medically.

2. PRECISION

Intra-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/ml)	18.79	128.67	507.22
S.D. (pg/ml)	1.96	5.26	27.44
C.V. (%)	10.4	4.1	5.4

Inter-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/ml)	18.36	128.52	505.77
S.D. (pg/ml)	1.72	12.5	48.55.44
C.V. (%)	9.4	9.7	9.6

3. ACCURACY/RECOVERY

Two serum samples were spiked with known conc. of Aldosterone. The Aldosterone values were measured and % of recovery was determined.

Initial Values (pg/ml)	Observed values (pg/ml)	Expected values (pg/ml)	Recovery (%)
Sample A Unspiked	45.30		
+ 51 pg/ml	119.1	96.3	123.7
+ 101 pg/ml	143.8	147.2	97.6
+ 203.80 pg/ml	227.5	249.1	91.3
Sample B Unspiked	130		
+ 51 pg/ml	209.4	181.0	115.7
+ 101 pg/ml	243.1	231.9	104.8
+ 203.80 pg/ml	307.5	333.8	92.1

3. LINEARITY

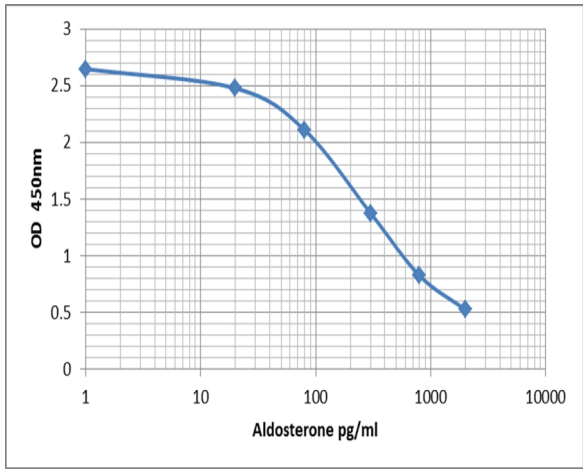
Two serum samples were diluted with Std. A. The Aldosterone values were measured and % of recovery was determined.

Initial Values (pg/ml)	Observed values (pg/ml)	Expected values (pg/ml)	Recovery (%)
Sample A Undiluted	395.1		
Dilution 1:2	198.6	197.6	100.5
Dilution 1:4	80.7	98.9	81.7
Dilution 1:8	44.2	49.5	89.5
Sample B Undiluted	414.2		
Dilution 1:2	206.7	207.1	99.8
Dilution 1:4	103.9	103.6	100.3
Dilution 1:8	56.7	51.8	109.5

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pg/ml)	Net Mean $A_{450\ nm}$	Calc. Conc pg/ml
A1, A2	**Std. A** (0 pg/ml)	2.65	
B1, B2	**Std. B** (20 pg/ml)	2.5	
C1, C2	**Std. C** (80 pg/ml)	2.1	
D1, D2	**Std. D** (300 pg/ml)	1.35	
E1, E2	**Std. E** (800 pg/ml)	0.82	
F1, F2	**Std. F** (2000 pg/ml)	0.52	
G1, G2	**Sample 1**	**1.84**	**138**

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.



ADI-ELISA4
A typical std. assay curve (do not use this for calculating sample values)

Species Reactivity

This kit has been designed and tested for human serum samples. Plasma samples have been used in other species (mouse and rat). ADI Aldosterone ELISA kit has been used to measure mouse and rat aldosterone in plasma and in cell culture medium.

Min L-J 2005, Circ. Res. 97: 434 – 442, used rat VSMC culture medium
Suzuki J, 2006 Arterioscler. Thromb. Vasc. Biol 26: 917 – 921, Mouse plasma
Kang Y-M, 2006 Circ. Res. In press, rat plasma aldosterone ELISA
Kang Y-M, 2006 Circ. Res. 99: 758 - 766 rat plasma aldosterone ELISA

PRINCIPLE OF THE TEST

Aldosterone ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with Streptavidin-Biotin-linked Aldosterone for a fixed and limited number of antibody binding sites. In the assay, the Aldosterone standard or samples sera are incubated with Aldosterone-HRP conjugate and anti-Aldosterone. In this solid-phase system, the antibody bound Aldosterone will remain on the well while unbound Aldosterone will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Aldosterone-HRP conjugate. After a short incubation, the enzyme reaction is stopped (blue color turns yellow) and the intensity of the color (yellow) is measured using an ELISA plate reader. The color is inversely proportional to the concentration of Aldosterone in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (10-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Aldosterone ELISA test is intended for *in vitro research* use only. The reagents contain prolcin-300 as preservative, TMB and sulfuric acid. Necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera 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).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of Reagents

Standards and control: All standards and controls are provided in ready-to-use solutions. Once opened, they can be kept at 4oC and used within 7-14 days. For long-term usage, it is recommended to keep them frozen at –20oC or below is suitable size aliquots. Avoid repeated freeze and thaw.

Wash buffer Concentrate (10X): Prepare 1X solution by diluting 1:10 (50 ml concentrate in 450 ml water). Store diluted stock at 4oC.

Preparation of Aldosterone-Avidin HRP Conjugate: The solution is provided as 50X stock. Prepare 10 ml for a full plate (200 ul each of the conjugate in 10 ml of assay buffer) or 1 ml for each strip (20 ul/ml of the diluent).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. HRP substrate should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Important: Prepare working solutions of the conjugate & wash buffer.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Save 1 well for blank and do not add anything. Pipet **50 ul stds. and samples** into appropriate wells.

2. Pipet **100 ul of avidin conjugate** solution (1:50) into each well except blank well. Mix gently for 5-10 seconds. Cover the plate and incubate at room temp. for **60 min on plate shaker** (approx 200 rpm).

3. Remove reaction mixture and **wash 3X** with wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Pipet **150 ul of TMB substrate Soln** into each well (blue color develops). Mix gently for 5-10 seconds. Cover the plate and incubate at room temp. for **10-15 min** on plate shaker or until calibrator A attains dark blue color for desired OD).

5. Stop the reaction by adding **50 ul of stop** solution to all wells**.** Mix gently for 5-10 seconds. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader within 15-20 min.

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^{o}C. Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

1. Calculate the mean absorbance of standards and duplicate and subtract the blank values.

2. Plot the concentration (X) of each reference standard against the mean absorbance on a semi log paper. Draw a point-to-point line through the mean of the duplicate point. If immunoassay software is used, a 4-parameter curve is recommended. Obtain the value of sample aldosterone by standard curve.

3. If serum samples (>2000 pg/ml) were diluted then the values must be multiplied by the dilution factor.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Aldosterone values beyond the standard curve may yield variable results. Samples containing >2000 pg/ml Aldosterone can be diluted with 0 standard (no more than 1:8 dilution) and retested. The results must be multiplied by dilution factor. Controls from other manufacturers may contain **serum preservatives (azide or merthiolate)** incompatible with ADI's ELISA reagents should not be used. Whenever laboratory data conflict with clinical findings or impressions, clinical judgment should be exercised and additional evaluation undertaken. Grossly hemolyzed or lipemic samples may give erroneous results.

EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range.
2. The following values can be used as preliminary guidelines until the laboratory establishes its own normal values:

   Unrestricted salt intake, seated position
   N=54 **Mean = 105 pg/ml**
   Expected range: 25-315 pg/ml.
   Unrestricted salt intake Urine (5-19 ug/24 hr).

PERFORMANCE CHARACTERISTICS

Specificity

The antibody used in this kit is very sensitive and specific for aldosterone. The following compounds were tested for crossreactivity of the assay: Aldosterone (100%), 11-deoxycorticosterone (1.1%). The following steroids showed negligible (0.001%) crossreactivity: Androsterone, Cortisone, 11-deoxycortisol, 21-deoxycortisol, dihydrotestosterone, Estradiol, estriol, estrone, and testosterone.

Sensitivity

The minimal detectable conc. of Aldosterone is estimated to be **15 pg/ml**. The minimal detectable conc. is defines as the concn. of Aldosterone, which corresponds to the absorbance, that is 2 S.D. smaller than the mean abs. Value of the zero std.