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DCM053-8
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ALDOSTERONE ELISA

for routine analysis

Direct immunoenzymatic determination of Aldosterone in human serum, human plasma or urine.

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO053

INTENDED USE

Eagle Biosciences Aldosterone ELISA Assay Kit is a competitive immunoenzymatic colorimetric method for quantitative determination of Aldosterone concentration in human serum, human plasma or urine. Aldosterone ELISA Assay Kit is intended for research use only.

1. CLINICAL SIGNIFICANCE

Aldosterone is a steroid hormone produced by the adrenal cortex in the adrenal gland, is the most potent mineralocorticoid in humans, it regulate sodium and potassium balance in the blood.

Aldosterone secretion appears to be stimulated primarily through the renin-angiotensin system. Acting on mineralocorticoid receptors (MR) on principal cells in the collecting ducts of the kidneys, it increases the permeability of their apical (luminal) membrane to potassium and sodium and activates their basolateral Na+/K+ pumps, stimulating ATP hydrolysis, reabsorbing sodium (Na+) ions and water into the blood, and excreting potassium (K+) ions into the urine. Aldosterone regulate plasma bicarbonate (HCO3-) levels and its acid/base balance.

Aldosterone is responsible for the reabsorption of about 2% of filtered sodium in the kidneys.

Plasma aldosterone levels normally vary with body position (upright>supine) and salt intake. Overall plasma aldosterone levels show a circadian rhythm which is similar to but less marked than cortisol, with peak levels in the early morning; about 75% of the daily production is secreted between 04:00 am and 10:00 am each day. Age-related levels tend to decline from fetal through adult life.

Abnormally high plasma aldosterone concentrations can occur in adenomas, glucocorticoid-responsive hyperaldosteronism, idiopathic.

Abnormally low aldosterone secretion occurs in a number of conditions including salt-wasting forms of congenital adrenal hyperplasia, nephropathy, and renal tubular acidosis.

2. PRINCIPLE

The principle of this Aldosterone ELISA Assay Kit follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in calibrators, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding site on the microwell plate. The washing steps remove unbound materials. After the washing step, the enzyme substrate (TMB) is added. The enzymatic reaction is terminated by addiction of the Stop Solution. The intensity of the color is inversely proportional to the concentration of Aldosterone in the sample. The absorbance is measured on a microtiter plate reader. A set of calibrators is used to plot a calibration curve from which the amount of Aldosterone in samples and controls can be directly read.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (6 vials, 1 mL each)

CAL0	REF DCE002/5306-0
CAL1	REF DCE002/5307-0
CAL2	REF DCE002/5308-0
CAL3	REF DCE002/5309-0
CAL4	REF DCE002/5310-0
CAL5	REF DCE002/5311-0

2. Aldosterone Control (1 vial, 1 mL)
Concentration of Control is lot-specific and is indicated on the Certificate of Analysis REF DCE045/5303-0

3. Conjugate (1 vial, 1 mL)
Aldosterone conjúgated with horseradish peroxidase (HRP) REF DCE002/5302-0

4. Coated Microplate (1 breakable microplate)
Microplate coated with anti aldosterone antibody)
REF DCE002/5303-0

5. Incubation Buffer (1 vial, 30 mL)
Phosphate buffer 50 mM pH 7.5; BSA 1 g/L; stabilizers
REF DCE001-0

6. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L REF DCE006/5306-0

7. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

8. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note

Store all reagents between 2-8°C in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This Aldosterone ELISA Assay Kit is intended for research use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents of the Aldosterone ELISA Assay Kit contain small amounts of Proclin 300^{R} as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This Aldosterone ELISA Assay Kit allows the determination of Aldosterone from 20 to 2000 pg/mL
- The treatment with natural or synthetic steroids can affect blood levels of aldosterone.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the Aldosterone ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all Aldosterone ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange Aldosterone ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested..
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_5)

Leave on a rotating mixer for at least 5 minutes before using.
The Calibrators are ready to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4	C_5
pg/mL	0	20	80	300	800	2000

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of Wash Buffer

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

6.3. Preparation of Diluted Conjugate

Prepare immediately before use.
Dilute the Conjugate 1:50 into Incubation buffer (e.g. 20 µL of Conjugate can be diluted to 1 mL with Incubation buffer). Mix gently for almost ten minutes on a rotating mixer.

6.4. Preparation of the Sample

The determination of Aldosterone can be performed in human serum, human plasma or in urine. Store the sample at -20°C if the determination is not performed on the same day of the sample connection.

For sample with concentration over 2000 pg/mL dilute the sample with zero Calibrator.
For Urine determination please see annex A.
The Control are ready to use.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Samples/ Control	Blank
Calibrator C_0-C_5	**50 µL**		
Samples/ Control		**50 µL**	
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate at +37°C for 1 hour. Remove the contents from each well; wash the wells 3 times with 300 µL of diluted wash solution.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 20 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Aldosterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_5) and of each sample.

8.2. Calibration curve

Plot the values of absorbance (Em) of the Calibrators (C_0-C_5) against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in pg/mL.

9. REFERENCE VALUES

Serum:		**pg/mL**	
Healthy Adult		Mean	Range
Early Morning, Supine		68.9	20-180
Upright, 2 Hours		109.2	30-400
24-Hour Urine:		**µg/day**	
Healthy Adult	Volume	Mean	Range
Urine	1650 mL	11.83	2-22

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate measurements (15x) of two different control sera in one assay. The within assay variability is ≤ 9.7%.

10.1.2. Inter Assay Variation

Between run variation was determined by replicate measurements (10x) of three different control sera in different lots of kits. The between assay variability is ≤ 11%.

10.2. Accuracy

The recovery of 0 - 300 - 800 - 2000 (pg/mL) of Aldosterone added to two patient samples gave an average value (±SE) of 103.94% ± 2.78%.

10.3. Sensitivity

The lowest detectable concentration of Aldosterone that can be distinguished from the Calibrator 0 is 7 pg/mL at the 95% confidence limit.

10.4. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Aldosterone	100.0%
11-Deoxicorticosterone	1.10%
Androsterone	< 0.001%
Cortisone	< 0.001%
11-Deoxicortisol	< 0.001%
21-Deoxicortisol	< 0.001%
Dihydrotestosterone	< 0.001%
Estradiol	< 0.001%
Estriol	< 0.001%
Estrone	< 0.001%
Testosterone	< 0.001%

10.5. Correlation with RIA

Diametra Aldosterone ELISA was compared to a commercially available Aldosterone RIA assay. 56 serum samples were tested. The linear regression curve was calculated:

$y = 1.03 x + 1.64$

$r^2 = 0.99$

y = Aldosterone Diametra Elisa Kit

x = Aldosterone Adaltis MAIA RIA Kit

14 urine samples were tested. The linear regression curve was calculated:

$y = 0.86 x + 12.53$

$r^2 = 0.92$

y = Aldosterone Diametra Elisa Kit

x = Aldosterone Adaltis MAIA RIA Kit

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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DiaMetra S.r.l. Headquater: Via Garibaldi, 18 – 20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039–02–2133354.
Manufactory: Via Pozzuolo 14, 06038 SPELLO (PG) Italy
Tel. 0039-0742–24851
Fax 0039–0742–316197
E-mail: info@diametra.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

Annex A

Sample Preparation: Urine
Precautions: Ethyl acetate is a volatile, flammable organic solvent. Conduct the evaporation step under a fume hood equipped with an explosion-proof exhaust fan. Avoid open flames, and do not pipet by mouth. The ethyl acetate must be of at least spectrophotometric grade.

1 Label one glass or polypropylene tube for each urine sample.
The tubes should have tight-fitting caps and be able to withstand centrifuging at 1500xg

2 Pipet **250 µL** of each urine sample into the appropriate tube.
If the sample is cloudy or if a precipitate has formed, first centrifuge the urine and work with the supernatant.

3 Hydrolysis: Add **25 µL** of 3.2 *N* HCl (not supplied) to every tube. Cap securely and incubate for 24 hours at room temperature *in the dark.*
3.2 *N* HCl can be made by adding 1.0 mL concentrated HCl (12*N*) to 2.75 mL distilled water. Do not add water to concentrated acid, since this may cause splattering.

4 Extraction: Add **2.5 mL** ethyl acetate (not supplied) to every tube. Cap securely.

5 Mix by gentle inversion for **60 minutes**.
Use a mechanical rotator set at 15–20 revolutions-per-minute.

6 Centrifuge for **5 minutes** at about 1500xg, to separate the two layers.
Any sample partially emulsified should be shaken vigorously and centrifuged again.

7 Evaporation: Transfer exactly **100 µL** of the upper (ethyl acetate) phase cleanly into one plain (uncoated) 12x75 mm polypropylene tube.
(Do not use polystyrene) Pipet directly to the bottom of the tube using a positive-displacement micropipet.
The remainder of the ethyl acetate phase may be retained for future use simply by freezing the extraction tube at –20°C;
it is not necessary to separate the ethyl acetate phase from the aqueous phase.

8 Evaporate to complete dryness under a gentle stream of nitrogen at 37°C.

9 Add **0.5 mL** of the Incubation buffer **REF DCE001-0** (auxiliary reagent) or saline solution NaCl 0.9%.
Thoroughly resuspend the extract by vortexing.

10 Transfer **50 µL of Resuspend to the well of coated microplate**

Proceed with the assay procedure, as described in the IFU (using the resuspended as normal sample)

Calculation Urine Samples: The result in "pg/mL" as read from the calibration curve must be multiplied by 100 to obtain the aldosterone concentration, in picograms per milliliter, of the original, unextracted urine sample.
Divide this figure by 1,000, then multiply by the total volume *in liters:,* to report the 24-hour aldosterone output in micrograms per day.
(A correction factor of 100 is used because the urine samples are twice diluted 1-in-10: first by extracting 0.25 mL urine into 2.5 mL ethyl acetate, then by reconstituting the residue of 0.1 mL in 0.5 mL of Incubation buffer (Saline Solution) and using 50 µL of it.
The addition of hydrochloric acid during the hydrolysis step has no effect on the dilution.

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation