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Instructions for use Aldosterone ELISA









Aldosterone ELISA

1. Introduction

1.1 Intended use

The **Aldosterone ELISA** is an enzyme immunoassay for the measurement of aldosterone in serum, plasma (EDTA-, heparin- or citrate plasma) and urine.

1.2 Summary and Explanation

The steroid hormone aldosterone is a potent mineral corticoid that is produced by the zona glomerulosa of the adrenal cortex in the adrenal gland. The synthesis and release are controlled by the renin-angiotensin-aldosterone system (RAAS)¹, as well as by plasma potassium concentration ², the pituitary peptide ACTH, and by the blood pressure via pressure sensitive baroreceptors in the vessel walls of nearly all large arteries of the body³. Aldosterone binds to mineralocorticoid receptors (MR) and triggers the transcription of hormone-responsive genes.

2. PRINCIPLE OF THE TEST

The Aldosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with a polyclonal rabbit antibody directed towards an antigenic site of the aldosterone molecule. Endogenous aldosterone of a sample competes with an aldosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of aldosterone in the sample.

3. WARNINGS AND PRECAUTIONS

- 1. This kit is for research use only. For professional use only.
- 2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- 3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- 4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C 8 °C in the sealed foil pouch and used in the frame provided.
- 5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- 6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- 7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- 8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- 9. Allow the reagents to reach room temperature (21 °C 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- 10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- 11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- 12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- 13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- 14. Do not use reagents beyond expiry date as shown on the kit labels.
- 15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- 16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- 17. Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
- 18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- 19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.

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- 20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- 21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from manufacturer.

4. REAGENTS

4.1 Reagents provided

Contents: 12 x 8 (break apart) strips; 96 wells; Wells coated with anti-aldosterone antibody

(polyclonal rabbit)

Standards and Controls - lyophilized

Cat. no.	Component	Concentration	Volume/ Vial
MS E-5201	STANDARD A	0 pg/ml	1.0 ml
MS E-5202	STANDARD B	20 pg/ml	1.0 ml
MS E-5203	STANDARD C	80 pg/ml	1.0 ml
MS E-5204	STANDARD D	200 pg/ml	1.0 ml
MS E-5205	STANDARD E	500 pg/ml	1.0 ml
MS E-5206	STANDARD F	1000 pg/ml	1.0 ml
MS E-5251	CONTROL 1	For control values and	1.0 ml
MS E-5252	CONTROL 2	ranges please refer to vial label or QC-Report.	1.0 ml

Conversion: 1 pg/ml corresponds to 2.77 pmol/l Contents: Contain non-mercury preservative.

see "Reagents Preparation"

MS E-5240 CONJUGATE Enzyme Conjugate - Ready to use Contents: Aldosterone conjugated to horseradish peroxidase,

Contain non-mercury preservative.

Volume: 1 x 20 ml

SA E-0055 SUBSTRATE Substrate Solution - Ready to use

Contents: Tetramethylbenzidine (TMB).

Volume: 1 x 25 ml

FR E-0080 STOP-SOLN Stop Solution - Ready to use

Contents: contains 0.5 M H₂SO₄

Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 14 ml

Hazards

identification:



H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

FR E-0030 WASH-CONC 40x Wash Solution - 40x concentrated

Volume: 1 x 30 ml See "Preparation of Reagents".

Note: Additional Standard A for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Scale paper or semi-logarithmic graph paper or software for data reduction

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- Optional: Reagents for determination of Aldosterone in urine (REF MS U-5200) Contents:
 - 1) *Release Reagent*, 1 vial, 3 ml, ready to use. Containing 1M HCl. Avoid contact with *Release Reagent*. It may cause skin irritation.
 - 2) Neutralization Buffer, 1 vial, 3 ml, ready to use. Containing Tris buffer, pH 8.5.
 - 3) Dilution Buffer, 2 vials, 25 ml each, ready to use. Containing PBS.
- Optional: Plastic tubes (e.g. 0.5 1.5 ml) for pre-treatment of urine samples

4.3 Storage Conditions

When stored at $2\,^{\circ}\text{C}$ to $8\,^{\circ}\text{C}$ unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vials with 1.0 ml deionized water and let stand for at least 10 minutes. Mix several times before use.

Note: The reconstituted standards are stable for 8 weeks at 2 °C to 8 °C.

For longer storage freeze - only once - at -20 °C.

Controls

Reconstitute the lyophilized content of the controls with 1.0 ml deionized water and let stand for at least 10 minutes. Mix several times before use.

Note: The reconstituted controls are stable for 8 weeks at 2 °C to 8 °C.

For longer storage freeze - only once - at -20 °C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml.

The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate plasma) and urine can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Serum / Plasma Samples

5.1.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred.

Plasma

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

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5.1.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (up to two months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.1.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard A* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example

- a) dilution 1:10: 10 µl sample + 90 µl Standard A (mix thoroughly)
- b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Standard A (mix thoroughly).

5.2 Urine Samples

Aldosterone concentration can also be determined from urine samples. However, urine samples must be pretreated before analysis. This will need additional reagents that are not included in this kit, but can be ordered separately (MS U-5200).

5.2.1 Sample Collection

First clean genital area with mild disinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container. Directly after collection, the urine should be centrifuged for 5 - 10 minutes (e.g. at 2,000 g) to remove cellular debris. Use supernatant for analyte quantification. The supernatant may be stored for up to 8 hours at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen at -20 °C. Thawed supernatant should be inverted several times prior to testing.

5.2.2 Protocol for Urine Sample Pre-treatment

- 1. Secure the desired number of vials (e.g. 0.5 1.5 ml plastic tubes; not included in this kit).
- 2. Dispense 25 µl of urine with new disposable tips into appropriate tubes.
- 3. Dispense 25 µl Release Reagent into each tube

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

- 4. Incubate over night at 2 °C to 8 °C.
- 5. Add **25 µl** *Neutralization Reagent* to each tube and mix thoroughly.
- 6. Add 400 µl Dilution Buffer to each tube and mix thoroughly

(This pre-treatment leads to a 1:19 dilution. Therefore the <u>dilution factor 19</u> has to be taken into account for calculation of the final concentration of the urine sample.)

7. Transfer **50** µl of pre-treated and diluted urine samples directly to the microtiter well and continue with step 3 of Test Procedure (Chapter 6.2).

5.2.3 Storage of pre-treated urine samples

Pre-treated and diluted urine samples should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying.

Samples held for a longer time (up to two months) should be frozen only once at -20 °C prior assay.

Thawed samples should be inverted several times prior to testing.

5.2.4 Specimen Dilution

If in an initial assay, an urine sample is found to contain more than the highest standard, the <u>pre-treated and diluted</u> urine sample can be further diluted with *Dilution Buffer* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account too.

Example:

a) dilution 1:10: 10 μ l pre-treated and diluted urine sample + 90 μ l Dilution Buffer (mix thoroughly) (final dilution factor = 19 x 10 = 190)

6. ASSAY PROCEDURE

6.1 General remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.

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- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

- 1. Secure the desired number of Microtiter wells in the frame holder.
- Dispense 50 μI of each Standard, Control and samples with new disposable tips into appropriate wells.

For urine samples dispense 50 μ l of the <u>pre-treated and diluted urine samples</u> (see chapter 5.2.2 Protocol for Urine Sample Pre-treatment, step 7).

- 3. Incubate for 30 minutes at room temperature.
- 4. Dispense **150** µI *Enzyme Conjugate* into each well.

 Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
- 5. Incubate for 60 minutes at room temperature.
- 6. Briskly shake out the contents of the wells. Rinse the wells
 - 5 x with 400 µl diluted Wash Solution per well (if a plate washer is used) or.
 - 5 x with 300 µI/well for manual washing.

Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

- 7. Add 200 µl of *Substrate Solution* to each well.
- 8. Incubate for 30 minutes at room temperature.
- 9. Stop the enzymatic reaction by adding 100 µl of Stop Solution to each well.
- 10. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the *Stop Solution*.

6.3 Calculation of Results

- 1. Calculate the average absorbance values for each set of standards, controls and samples.
- 2. Using scale paper or semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
- 3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
- 4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
- 5. The concentration of the **serum / plasma samples** can be read **directly** from this standard curve. For **urine samples** the concentration read from the standard curve, has to be **multiplied** with the **dilution factor 19** (see chapter 5.2.2).
- 6. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 1000 pg/ml. For the calculation of the concentrations this dilution factor has to be taken into account too.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard A (0 pg/ml)	2.11
Standard B (20 pg/ml)	1.90
Standard C (80 pg/ml)	1.55
Standard D (200 pg/ml)	1.15
Standard E (500 pg/ml)	0.76
Standard F (1000 pg/ml)	0.54

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6.4 Final Calculation for Urine Samples

Calculate the 24 hours excretion for each urine sample: $\mu g/24 h = \mu g/1 \times 1/24 h$

Example:

Concentration for urine sample read from the standard curve = 500 pg/mlResult after correction with the dilution factor 19 = 9500 pg/ml

 $9500 \text{ pg/l} / 1000 = 9.5 \mu\text{g/l}$

Total volume of 24 h-urine = 1.3 l (example)

 $9.5 \,\mu g/l \times 1.3 \,l/24 \,h$ = 12.35 $\mu g/24 \,h$

7. EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

7.1 Serum/Plasma

In a study conducted with **EDTA plasma samples** of apparently normal healthy adults, using the Aldosterone ELISA the following values are observed:

Healthy Adults	n	Mean (pg/ml)	Median (pg/ml)	2.5 th - 97.5 th percentile (pg/ml)	Range(min - max) (pg/ml)
Supine position	60	56.14	39.71	14.21 - 156.47	8.58 - 272.30
Upright position	60	77.48	58.00	13.37 - 233.55	12.87 - 358.50

These values are also valid for serum, heparin plasma and citrate plasma.

These results correspond well to published reference ranges 8,9.

In a study conducted with apparently normal healthy adults, using the Aldosterone ELISA and the Renin ELISA the following *Aldosterone-Renin Ratios* were determined in plasma:

Ratio Aldosterone Renin (pg/ml / pg/ml)

	n	Mean	Median	2.5 th - 97.5 th Percentile
Healthy Adults	89	8.68	5.30	0.52 - 37.83

7.2 Urine Samples

In a study conducted with **urine samples** of apparently normal healthy adults, using the Aldosterone ELISA the following values are observed:

	n	Mean (µg/24h)	Median (µg/24h)	2.5 th - 97.5 th percentile (µg/24h)	Range (min - max) (µg/24h)
Healthy Adults	8	11.34	9.40	3.31 - 25.09	3.06 - 27.17

These results correspond well to published reference ranges 8.

8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC Report added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

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9. PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

For <u>serum and plasma</u> the range of the assay is between 7.75 pg/ml - 1000 pg/ml. For <u>urine</u> the range of the assay is between 9.81 pg/ml - 1000 pg/ml.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Substance	Cross Reactivity (%)
3 β, 5 a Tetrahydroaldosterone	17.2 %
3 β, 5 β Tetrahydroaldosterone	0.12 %

The following steroids were tested but cross-reacted at less than 0.1%: androstenedione, androsterone, corticosterone, cortisol, cortisone, 11-deoxycortisol, DHEA, estradiol, estriol, estrone, 17-hydroxyprogesterone, prednisolone, prednisone, progesterone and testosterone.

9.3 Sensitivity

The <u>analytical sensitivity</u> of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the *Standard A* (Std. A) and was found to be < 5.7 pg/ml.

	Serum / Plasma	Urine
The Limit of Blank (LoB)	7.75 pg/ml	9.81 pg/ml
The Limit of Detection (LoD)	12.07 pg/ml	16.94 pg/ml
The Limit of Quantification (LoQ)	14.78 pg/ml	23.66 pg/ml

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
Serum 1	40	61.93	4.63
Serum 2	40	247.50	2.02
Serum 3	40	560.33	1.80
Urine 1	40	79.12	5.91
Urine 2	40	229.99	4.85
Urine 3	40	495.82	5.09

9.4.2 Inter Assay

The within assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	80	61.93	10.06
2	80	547.50	5.07
3	80	560.33	4.65
Urine 1	80	79.12	13.92
Urine 2	80	229.99	9.44
Urine 3	80	495.82	9.65

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9.5 Recovery

Samples have been spiked by adding aldosterone solutions with known concentrations in a 1:1 ratio. The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous aldosterone + added aldosterone) / 2; because of a 1:2 dilution of serum with spike material).

	Serum 1	Serum 2	Serum 3	Urine 1	Urine 2	Urine 3
Concentration [pg/ml]	94.6	211.6	585.3	70.59	232.55	516.52
Average Recovery	91.3	107.4	98.2	102.1	99.9	95.1
from	85.1	104.5	91.3	97.4	92.7	91.0
Range of Recovery [%] to	96.2	110.9	102.8	108.0	108.2	99.1

9.6 Linearity

	Serum 1	Serum 2	Serum 3	Urine 1	Urine 2	Urine 3
Concentration [pg/ml]	296.3	406.0	631.2	569.8	655.5	683.7
Average Recovery	101.2	101.6	95.5	98.2	102.7	105.3
from	96.7	93.6	90.6	95.4	91.0	101.8
Range of Recovery [%] to	105.6	109.4	98.6	105.5	111.3	110.6

10. LIMITATION OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

The following substances have no influence on the assay results up to the below stated concentrations.

	Serum	Urine		
	Concentration up to			
Haemoglobin	6 m	g/ml		
Bilirubin	0.4 mg/ml			
Triglyceride	20 mg/ml			
Cholesterol	2.84 mg/ml			
Total protein	120 mg/ml			
Glucose	10 mg/ml			
Creatinine	0.05 mg/ml 5 mg/ml			

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of aldosterone in a sample.

10.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

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11. REFERENCES / LITERATURE

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△ For updated literature or any other information please contact your local supplier.

Symbols:

+ <u>2</u>	Storage temperature	***	Manufacturer	Σ	Contains sufficient for <n> tests</n>
\subseteq	Expiry date	LOT	Batch code		
\bigcap i	Consult instructions for use	CONT	Content		
\triangle	Caution	REF	Catalogue number	RUO	For research use only!

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