Instructions for Use

IBL International GmbH
Flughafenstraße 52 a
22335 Hamburg

Tel. +49 (0) 40 53 28 91-0
Fax +49 (0) 40 53 28 91-11

IBL@tecan.com
www.tecan.com/ibl

Aldosterone ELISA

Enzyme immunoassay for the quantitative determination of Aldosterone in human serum, plasma (EDTA, Li-heparin or citrate plasma) and urine.

REF **RE52301**

Σ **96**

2°C 8°C

EU: IVD CE

IBL International GmbH
Flughafenstrasse 52a
22335 Hamburg, Germany

Please use only the valid version of the Instructions for Use provided with the kit.

1 INTENDED USE

The **Aldosterone ELISA** is a manual enzyme immunoassay for the **quantitative** measurement of aldosterone in human serum or plasma (EDTA, Li-heparin or citrate plasma) or urine.

For *in vitro* diagnostic use. For laboratory professional use.

The device is **intended to be used** as an aid to diagnosis of primary and secondary aldosteronism.

The device is **not intended** for the diagnosis of adenomas.

1.1 Scientific Validity

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,[2,12] the pituitary peptide ACTH, and by the blood pressure via pressure sensitive baroreceptors in the vessel walls of nearly all large arteries of the body.[3,12]. Aldosterone binds to mineralocorticoid receptors (MR) and triggers the transcription of hormone-responsive genes. In consequence, aldosterone increases the blood pressure by reabsorption of sodium and water from the distal tubules of the kidney into the blood, secretion of potassium into the urine, and elevation of circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension. Aldosterone activity is reduced in Addison's disease and increased in Conn's syndrome.
Primary hyperaldosteronism, which may be caused by aldosterone-secreting adrenal adenoma/carcinomas or adrenal cortical hyperplasia, is characterized by hypertension accompanied by increased aldosterone levels, hypernatremia, and hypokalemia. Secondary hyperaldosteronism (e.g. in response to renovascular disease, salt depletion, potassium loading, cardiac failure with ascites, pregnancy, Bartter's syndrome) is characterized by increased aldosterone levels and increased plasma renin activity.[4,5,8,9,10,11,13]

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

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.[6]

In addition, pharmacological modulation of nuclear hormone receptors is a common strategy for the treatment of cardiovascular disease.[7] Therefore, determining the effects of such treatments on the RAAS is of increasing value in evaluating the safety and efficacy of new therapeutics.

In addition, obese subjects often exhibit hyperaldosteronism, with increased salt sensitivity of blood pressure (BP). Systemic RAS, and aldosterone/MR activation plays a key role in the development of hypertension and organ damage in obesity.[14]

In summary, the precise and accurate measurement of serum aldosterone by enzyme immunoassay can be an important adjunct to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

2 PRINCIPLE OF THE TEST

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

The microtiter wells are coated with a monoclonal antibody (mouse) directed towards a unique antigenic site of the aldosterone molecule.

During the first incubation, the aldosterone in the added sample competes with the added enzyme conjugate, which is aldosterone conjugated to horseradish peroxidase, for binding to the coated antibody.

After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

- This kit is for *in vitro* diagnostic use only. For laboratory professional use only.
- Before starting the assay, read the instructions for use completely and carefully.
  Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- Do not mix or use components from kits with different lot numbers. It is advised not to interchange 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.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Do not reuse microtiter wells.
- Reagents of other manufacturers must not be used together with the reagents of this test kit.
- All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact IBL.
- Microbial contamination of reagents or samples may give false results.
- Allow the reagents to reach room temperature (20°C to 26°C) before starting the test. Temperature will affect the optical density readings of the assay.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- 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 coloured. Do not pour reagents back into original vials as reagent contamination may occur.

General precautions

- Follow good laboratory practice and safety guidelines.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
- Wear lab coats and disposable latex gloves when handling samples and reagents and where necessary safety glasses.

Biohazard information

- 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. However, no known test method can offer total assurance that no infectious agent is present.
- The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
- Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.
- All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
- Handling must be done in accordance with the procedures defined by appropriate national biohazard and safety guideline or regulation. Waste must be discarded according to local rules and regulations.

Information to chemical hazards and hazard classification

- Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
- Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately carefully and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
- This product does not contain substances which have carcinogenic, mutagenic or toxic for reproduction (CMR) properties.

All reagents of this test kit do NOT contain hazardous substances in concentrations to be declared, a classification and labelling is not required.

For detailed information, please refer to the Safety Data Sheet, which is available upon request.

4 MATERIALS

4.1 Materials provided with the kit

Symbol	Quantity	Description	Preparation
MTP	12 x 8 wells (break apart)	**Microtiter Plate** Coated with anti-aldosterone antibody (monoclonal).	Ready to use
ENZCONJ	1 x 14 mL	**Enzyme Conjugate *** Aldosterone conjugated to horseradish peroxidase; Colored red.	Ready to use
CAL A-F LYO	1x 6 x	**Standard A - F *** Concentrations: 0; 20; 80; 200; 500; 1000 pg/mL Conversion: 1 pg/mL = 2.77 pmol/L *Calibrated against the following reference material: Certified reference material Cerilliant A-096*	Lyophilized; *See "Reagent Preparation".*
CONTROL - LYO CONTROL + LYO	1 x 2 x	**Controls*** *For control values and ranges please refer to vial label or Certificate of Analysis.*	Lyophilized; *See "Reagent Preparation".*
WASHBUF CONC	1 x 30 mL	**Wash Solution, 40X concentrate ***	*See "Reagent Preparation".*
TMB SUBS	1 x 14 mL	**Substrate Solution** Contains 3,3',5,5'-tetramethylbenzidine (TMB). *Keep away from direct sun light.*	Ready to use
TMB STOP	1 x 14 mL	**Stop Solution** Contains 0.5 M H_2SO_4. *Avoid contact with the stop solution. It may cause skin irritations and burns.*	Ready to use
	1 x	**Instructions for Use**	
	1 x	**Certificate of Analysis (CoA)**	
			***** Contains non-mercury preservative.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing microtiter plate wells
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction
- **Optional**: Reagents for determination of **Aldosterone in urine** (REF RE52307)
  Contents:
  1) ***Release Reagent***, 1 vial, 3 mL, ready to use. Containing 1 M 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 and Stability of the Kit

Unopened kits and reagents as well as **opened reagents** must be stored at 2°C to 8°C.

The microtiter plate contains snap-off strips. Do not open the pouch of the wells until it reaches room temperature. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch including the desiccant and used in the plate frame provided. Once the foil bag has been opened, care must be taken to close it tightly again. Once opened, reagent vials must be closed tightly again.

	Storage Temperature	Stability
Unopened kits and unopened reagents	2°C to 8°C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2°C to 8°C	8 weeks (For reconstituted reagents refer to "4.4 Reagent Preparation".)

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20°C to 26°C) prior to use.

Standards

Reconstitute the lyophilized contents of each standard vial with 1.0 mL distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Stability after reconstitution:	at 2°C to 8°C	8 weeks
	at -20°C (in aliquots), freeze only once	12 months

Controls

Reconstitute the lyophilized content of each control vial with 1.0 mL distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Stability after reconstitution:	at 2°C to 8°C	8 weeks
	at -20°C (in aliquots)), freeze only once	12 months

Wash Solution

Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL distilled water to a final volume of 1200 mL.

Stability after dilution:	at 20°C to 26°C	1 week

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

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

5 SAMPLE COLLECTION, STORAGE AND PREPARATION

The following sample material can be used in this test:

human serum or plasma (EDTA plasma, lithium heparin plasma or citrate plasma) and **urine**
Samples containing sodium azide should not be used in the assay.

In general, it should be avoided to use hemolytic, icteric, or lipemic samples. For further information refer to chapter "*Interfering Substances*".

5.1 Serum / Plasma Samples

5.1.1 Sample 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. Patients receiving anticoagulant therapy may require increased clotting time.

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

Whole blood should not be frozen before centrifugation.

5.1.2 Samples Storage

Samples must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once.
Thawed samples must be inverted several times prior to testing.

Stability	at 2°C to 8°C	5 days
	at -20°C (in aliquots)	up to 12 months

5.1.3 Sample Preparation

Serum and plasma samples can be assayed without additional preparation.

5.1.4 Sample Dilution

If in an initial assay, a serum or plasma sample is found to contain more than the highest standard, it 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: dilution 1:10: 10 µL sample + 90 µL *Standard A* (mix thoroughly)

5.2 Urine Samples

Aldosterone concentration can also be determined from urine samples. However, urine samples must be pre-treated before analysis. This will need additional reagents that are not included in this kit but can be ordered separately (REF RE52307).

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.

Since aldosterone secretion follows a circadian rhythm, an urine collection is recommended in a special cooled container over a full 24-hour period (24-hour urine).

Directly after collection, the urine should be centrifuged for 5 - 10 minutes (e.g. at 2000 *g*) to remove cellular debris.

Use supernatant for analyte quantification.

5.2.2 Samples Storage

Urine supernatant must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once. Thawed supernatant must be inverted several times prior to testing.

Stability of urine supernatant	at 2°C to 8°C	7 days
	at -20°C (in aliquots)	7 days

5.2.3 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 overnight 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 dilution factor 19 has to be taken into account for calculation of the final concentration of the urine sample.)
7. Transfer **100 µL of pre-treated and diluted urine samples** directly to the microtiter well and continue with step 3 of Test Procedure (Chapter 6.2).

5.2.4 Storage of Pre-treated Urine Samples

Samples must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once. Thawed samples must be inverted several times prior to testing.

Stability of pre-treated and diluted urine samples	at 2°C to 8°C	7 days
	at -20°C (in aliquots)	7 days

5.2.5 Urine Sample Dilution

If in an initial assay, an urine sample is found to contain more than the highest standard, the pre-treated and diluted 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: dilution 1:10: 10 µL pre-treated and diluted urine sample + 90 µL *Dilution Buffer* (mix thoroughly)
(final dilution factor = 19 x 10 = 190)

6 ASSAY PROCEDURE

6.1 Procedural Notes

- All reagents and samples must be allowed to come to room temperature (20°C to 26°C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid carry-over.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps must be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in chapter “Test Procedure”.
- 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.
- **Important note to wash procedure:**
  Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:**
  Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

6.2 Test Procedure

Each run must include a standard curve.

The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.

The given test procedure describes manual processing.

1. Secure the desired number of microtiter wells in the frame holder.
2. Pipette **100 µL** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells. For urine samples dispense **100 µL** of the pre-treated and diluted urine samples (see chapter 5.2.2 *Protocol for Urine Sample Pre-treatment*, step 7).
3. Incubate for **30 minutes** at room temperature.
4. Add **100 µL** ***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. Wash the wells as follows:
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted *Wash Solution* per well.
   If an automated plate washer is used:
   Rinse the wells **3 times** with **400 µL** diluted *Wash Solution* per well.
   At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
7. Pipette **100 µL** of ***Substrate Solution*** to each well.
8. Incubate for **30 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
10. Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** 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. The concentration of the **serum / plasma samples** can be read **directly** from the 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).
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and patient sample must be taken. If the two values deviate substantially from one another, IBL recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted and re-assayed as described in “Test Procedure”, or must be reported as > 1000 pg/mL. For the calculation of the concentrations, this dilution factor must be considered.
4. Automated method:
   The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. Manual method:
   Using linear or semi-logarithmic graph paper, construct a standard curve by plotting the (mean) OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis. Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

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 Density (450 nm)
Standard A (0 pg/mL)	2.040
Standard B (20 pg/mL)	1.634
Standard C (80 pg/mL)	0.966
Standard D (200 pg/mL)	0.516
Standard E (500 pg/mL)	0.223
Standard F (1000 pg/mL)	0.130

6.4 Final Calculation for Urine Samples

Calculate the 24 hours excretion for each urine sample: µg/24 h = **µg/L x L/24 h**
Example: Concentration for urine sample read from the standard curve = 500 pg/mL

Result after correction with the dilution factor 19 = 9500 pg/mL

9500 pg/mL／1000 = 9.5 µg/L
Total volume of 24 h-urine = 1.3 L (example)
9.5 µg/L × 1.3 L/24 h = **12.35 µg/24 h**

7 REFERENCE VALUES

It is strongly recommended that each laboratory determine its own reference 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)	2.5th - 97.5th 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, 15]

In a study conducted with apparently normal healthy adults, using the Aldosterone ELISA (RE52301) and the Renin ELISA (RE53321) the following ***Aldosterone-Renin Ratios*** were determined in plasma:

Ratio Aldosterone-Renin

	n	Mean (pg/mL⁄pg/mL)	Median (pg/mL⁄pg/mL)	2.5th - 97.5th Percentile (pg/mL⁄pg/mL)
Healthy Adults	89	8.68	5.30	0.52 - 37.83

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

These results correspond well to published reference ranges.[16]

7.2 Urine Samples

In a study conducted with **urine samples (24-hours urine)** of apparently normal healthy adults, using the Aldosterone ELISA the following values are observed:

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

These results correspond well to published reference ranges.[8]

The results alone should not be the only reason for any therapeutic consequences. The results must be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration 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 Quality Control Laboratory are stated in the Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.

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

Apply appropriate statistical methods for analyzing control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, patient 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 IBL directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Specificity of Antibodies (Cross-Reactivity)

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

Substance	Conc. Range of Spiked Substance	Mean Cross-Reactivity (%)
11-Deoxy Cortisol	10 - 1000 ng/mL	0.01
17-OH Progesterone	1.2 - 120 ng/mL	0.00
21-OH Progesterone	3.5 - 350 ng/mL	0.04
Androstenedione	0.2 – 22 ng/mL	0.0
Androsterone	10 - 1000 ng/mL	0.00
BSA	1 - 100 mg/mL	0.00
Cholesterol	0.5 - 50 mg/mL	0.00
Corticosterone	0.5 - 50 ng/mL	0.15
Cortisol	8 - 800 ng/mL	0.0
Cortisone	16 - 1600 ng/mL	0.01
Creatinine	50 - 5000 µg/mL	0.00

Substance	Conc. Range of Spiked Substance	Mean Cross-Reactivity (%)
DHEA	0.5 - 50 ng/mL	0.03
DHEA-S	300 - 30000 ng/mL	0.00
Estradiol	0.02 - 2 ng/mL	0.01
Estriol	1.5 - 150 ng/mL	0.00
Estrone	0.01 - 1 ng/mL	0.05
Glucose	1 - 100 mg/mL	0.00
Prednisolone	35 - 3500 ng/mL	0.00
Prednisone	35 - 3500 ng/mL	0.00
Pregnenolone	35 - 3500 ng/mL	0.00
Progesterone	42.2 - 4220 ng/mL	0.00
Testosterone	0.01 - 1 ng/mL	0.00

9.2 Sensitivity

	Serum	Urine
Limit of Blank (LoB)	5.359 pg/mL	
Limit of Detection (LoD)	7.374 pg/mL	8.902 pg/mL
Limit of Quantification (LoQ)	10.647 pg/mL	15.665 pg/mL
Measuring range	7.374 pg/mL – 1000 pg/mL	8.902 – 1000 pg/mL
Linear range	16.37 pg/mL – 1000 pg/mL	23.73 – 1000 pg/mL

9.3 Reproducibility

9.3.1 Within-run Precision

The within-run precision was determined with 4 patient samples covering the complete measuring range in 5 independent runs within 5 days in 5 replicates per run. CV was calculated as mean CV of 5 runs.

Serum	n	Mean (pg/mL)	CV (%)
1	5	55.66	6.5
2	5	88.88	6.7
3	5	325.82	3.8
4	5	709.07	4.5

Urine	n	Mean (pg/mL)	CV (%)
1	5	88.47	4.3
2	5	167.59	6.4
3	5	343.98	5.5
4	5	634.55	5.9

9.3.2 Between-run Precision

The between-run variation was determined with 4 samples. The 4 samples are measured in 5 days with 5 replicates per run. 25 data points are generated per sample (5 replicates x 5 runs = 25 data points).

Serum	n	Mean (pg/mL)	CV (%)
1	25	55.66	7.4
2	25	88.88	9.8
3	25	325.82	6.5
4	25	709.07	9.6

Urine	n	Mean (pg/mL)	CV (%)
1	25	88.47	8.2
2	25	167.59	7.3
3	25	343.98	7.5
4	25	634.55	8.3

9.3.3 Between-lot Precision

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots.

Serum	n	Mean (pg/mL)	CV (%)
1	18	56.78	8.8
2	18	95.41	7.7
3	18	309.52	8.1
4	18	637.70	9.4

9.4 Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Serum 1	Serum 2	Serum 3	Serum 4	Urine 1	Urine 2	Urine 3	Urine 4
Concentration [pg/mL]		186.56	282.32	445.44	589.33	56.28	108.41	215.98	240.38
Average Recovery [%]		87.7	90.5	93.5	94.5	108.4	105.4	96.9	111.6
Range of Recovery [%]	**from**	86.0	87.9	92.6	90.9	106.8	104.0	88.8	108.8
	to	89.5	94.2	95.5	98.2	110.1	108.0	104.6	113.8

9.5 Linearity

Samples containing different amounts of analyte were serially diluted. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Serum 1	Serum 2	Serum 3	Serum 4	Urine 1	Urine 2	Urine 3	Urine 4
Concentration [pg/mL]		85.90	328.62	514.73	537.69	96.87	127.00	379.70	592.90
Average Recovery [%]		111.0	106.2	96.8	98.5	100.3	87.6	90.5	108.9
Range of Recovery [%]	**from**	108.5	102.2	93.2	88.0	90.7	87.6	85.8	106.5
	to	112.3	108.9	101.8	107.7	110.8	87.7	93.5	110.2

10 LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use 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

Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

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

10.3 High-Dose Hook Effect

"High-Dose Hook Effect" is not detected up to 20 000 pg/mL of aldosterone.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer’s instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. If there is any doubt or concern regarding a result, please contact IBL.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11.4 Reporting of Serious Incident

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

12 LITERATURE

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Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

IBL International GmbH
Flughafenstrasse 52a
22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@tecan.com
www.tecan.com/ibl

Always there for you