Human Aldosterone ELISA Kit

Enzyme Immunoassay for the quantification of human Aldosterone in serum, plasma and urine.

Catalog number: ARG80627

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INTRODUCTION

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), as well as by plasma potassium concentration, 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. 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.

Measurement of aldosterone levels in serum in conjunction with plasma renin levels (aldosterone/renin-ratio; ARR) can be used to differentiate between primary and secondary aldosteronism.

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

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.

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

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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. An antibody specific for aldosterone has been pre-coated onto a microtiter plate. The microtiter wells are coated with a polyclonal rabbit antibody directed towards an antigenic site of the aldosterone molecule. Endogenous aldosterone of a patient 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 color developed is inversely proportional to the concentration of aldosterone in the patient sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Standard 0-5 (0, 20, 80, 200, 500, 1000 pg/ml)	6 vials	4°C, Lyophilized
Control low (80.70 pg/ml, acceptable range: 44.39-117.0 pg/ml)	1 vial	4°C, Lyophilized
Control high (573.7 pg/ml, acceptable range: 315.5-831.9 pg/ml)	1 vial	4°C, Lyophilized
HRP conjugated Aldosterone	20 ml (ready to use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	25 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
- Optional: For urine sample preparation:
  - (a) Release Reagent: 1M HCl
  - (b) Neutralization Buffer: Tris buffer, pH8.5
  - (c) Dilution Buffer: PBS

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 EDTA, heparin or citrate as an anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection. Specimens should be capped and may be stored for up to 4 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.

Urine Samples - Aldosterone concentration can also be determined from urine samples. However, urine samples **must** be pre-treated before analysis.

Sample Collection

Clean genital area with mild disinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container. After collection, the urine should be centrifuged for 5 - 10 minutes at 2,000xg to remove cellular debris. Collect the supernatant for analytic quantification. The supernatant could be stored for up to 8 hours at 2 °C - 8 °C. For longer storage, aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. The thawed supernatant should be inverted several times before testing.

Protocol for urine sample pre-treatment:

(a) Secure the desired number of vials (e.g. 0.5- 1.5 mL plastic tubes; not included in this kit).

(b) Dispense 25 µL of urine with new disposable tips into appropriate tubes.

(c) Dispense 25 µL Release Reagent into each tube. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

(d) Incubate over night at 2 – 8 °C.

(e) Add 25 µL Neutralization Reagent to each tube and mix thoroughly.

(f) 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.)

(g) Transfer 50 µL of pre-treated and diluted urine samples directly to the microtiter well and continue with Assay Procedures.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. The diluted 1X wash buffer is stable for 2 weeks at room temperature.
- **Standards and controls**: Reconstitute with 1.0 mL deionized water and let stand for at least 10 minutes. Mix several times before use.

  Note: The reconstituted standards and controls are stable for 8 weeks at 2-8°C. For longer storage aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles. It is acceptable to freeze-thaw only once.
- **Samples**: If the initial assay found samples contain Aldosterone higher than the highest standard, the samples can be diluted and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  - **Serum and plasma sample:** Dilute the samples with **Standard 0**.

    a) Dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly).

    b) Dilution 1:100: 10 µL 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).
  - **Pretreated and diluted urine sample:** Dilute with **Dilution Buffer**:

    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)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of standards, controls and samples (urine samples must be pre-treated and diluted before assay) in duplicate into sample wells. Incubate for 30 minutes at RT.
3. Add 150 µl of HRP conjugated Aldosterone into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step. Incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (400 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 200 µl of TMB mixture to each well. Incubate for 30 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately. It is recommended to read the wells within 10 minutes after adding Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear 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 IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	0	1	2	3	4	5
Aldosterone (pg/ml)	0	20	80	200	500	1000
Aldosterone (pmol/L)	0	55.4	221.6	554	1385	2770
Conversion	Aldosterone (pg/ml) x 2.77 = Aldosterone (pmol/L)					

** Conversion: 1 pg/mL corresponds to 2.77 pmol/L.

6. 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

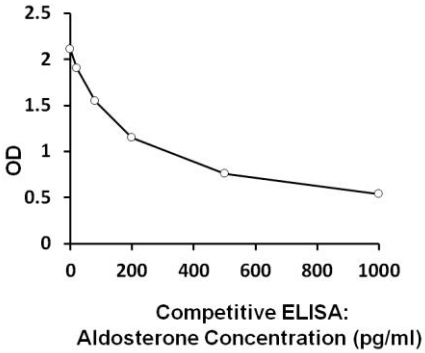
9500 pg/mL / 1000 = 9.5 µg/L

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

9.5 µg/L × 1.3 L/24 h = **12.35 µg/24 h**

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.



QUALITY ASSURANCE

Acceptable Range of Controls

Control	Mean (pg/ml)	Acceptable Range (pg/ml)
Low	80.70	44.39-117.0
High	573.7	315.5-831.9

Specificity

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

3 β, 5 α Tetrahydroaldosterone: 17.2 %

3 β, 5 β Tetrahydroaldosterone: 0.12 %

Prednisolone: 0.017 %

Cortisol: < 0.003 %

11-Deoxycortisol: < 0.003 %

Progesterone: < 0.003 %

Testosterone: < 0.002 %

Androstenedione: < 0.002 %

Assay Range

5.7 pg/ml - 1000 pg/ml

Sensitivity

The minimum detectable dose (MDD) of Aldosterone was found to be < 5.7 pg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.9% and inter-assay precision was 10.15%.

Recovery

92.4-114.8%

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.