Aldosterone ELISA

For the quantitative determination of Aldosterone in serum, plasma, and urine.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 11-AD2HU-E01

Size: 96 wells

Version: RUO-6.0 - ALPCO 2.0

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

Intended Use

The Aldosterone ELISA is for the direct quantitative determination of Aldosterone in human serum, plasma, and urine by an enzyme immunoassay. For research use only. Not for use in diagnostic procedures.

Principle of the Assay

The aldosterone ELISA is a competitive immunoassay. Competition occurs between aldosterone present in calibrators, controls, and samples and an enzyme-labeled antigen (HRP conjugate) for a limited number of antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB (enzyme) substrate is added and reacts with HRP to form a blue-colored product that is inversely proportional to the amount of aldosterone present. The enzymatic reaction is terminated by addition of the stop solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of aldosterone in samples and controls can be directly read.

Procedural Cautions and Warnings

1. This kit is intended for research use and *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and samples. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where samples or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiration date stated on the label.
5. If the kit reagents are visibly damaged, do not use the kit.
6. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended that all customers prepare their own control materials or serum/plasma/urine pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and the obtained values must fall within the acceptable ranges, as stated in the quality control certificate ; a failed control result might indicate improper procedural technique or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

14. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it must not be used.
15. Do not use grossly hemolyzed, lipemic, icteric, or improperly stored serum or plasma. Do not use improperly stored urine.
16. Samples or controls containing azide or thimerosal are incompatible with this kit. They may lead to false results.
17. Sample values above the measuring range of the kit should be reported as > 1000 pg/mL. If further dilution and retesting is required, only Aldosterone Serum and Plasma Diluent may be used to dilute serum and plasma samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stop solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate well, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the microplate wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

Safety Cautions and Warnings

Potential Biohazardous Material

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood samples. All human samples should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

Chemical Hazards

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stop Solution (contains sulfuric acid). If you come into contact with any of these reagents, wash with plenty of water and refer to the SDS for additional information.

Sample Collection and Storage

Serum: Approximately 0.2 mL of serum is required per duplicate determination. Collect 4 – 5 mL of blood into an appropriately-labeled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum layer into a new storage tube. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if analyses are to be done later.

Plasma: Approximately 0.2 mL of plasma is required per duplicate determination. Collect 4 – 5 mL of blood into appropriately-labeled EDTA plasma tube. Centrifuge at room temperature and carefully transfer the plasma into a new storage tube. Store at 2-8°C for up to 24 hours or at -10°C or lower if analyses is to be performed later.

Urine: Approximately 0.05 mL of urine is required for duplicate determination. Collect urine into a sample collection container over a 24-hour period (24-hour urine). Record the total volume (in mL) collected over 24 hours as this is required to calculate the level of aldosterone in urine samples. Urine samples may be stored at 2-8°C for up to 7 days.

Consider all human samples as possibly biohazardous materials and take appropriate precautions when handling.

Sample Pretreatment

Serum and plasma: Serum and plasma are loaded directly into the microplate wells; no sample pretreatment is necessary.

Urine: All urine samples must be diluted 1:50 in Urine Diluent on the day of the test. Follow the sample pre-treatment procedure as stated below for each sample that is to be tested. Do not store diluted urine samples.

1. Pipette 0.98 mL of Urine Diluent into a new polypropylene microcentrifuge tube.
2. Pipette 20 µL of the urine sample into the tube from step 1.
3. Close the tube and label it with sample identification information.
4. Mix the contents of the tube by vortexing.

Note: Different volumes of the Urine Diluent and urine sample may be used provided that the required 1:50 ratio is maintained (1 part urine sample to 49 parts Urine Diluent).

Consider all human samples as possibly biohazardous material.

Reagents and Equipment Needed but not Provided

1. Calibrated single-channel pipettes to dispense 20 µL (urine only), 50 µL, and 980 µL (urine only).

2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL, 150 µL, and 350 µL (if washing manually).
3. Automated microplate washer (recommended).
4. Disposable pipette tips
5. Distilled or deionized water
6. Plate shaker:
    a. Orbital shaker (3mm diameter) set to 600 rpm or
    b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations / minute.
7. Calibrated microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater.
8. Polypropylene tubes for sample pre-treatment (for urine samples only)
9. Urine Diluent – Required for the dilution of urine samples before assaying. Must be ordered separately.
10. Serum/Plasma Diluent – Required if high samples (> 1000 pg/mL) are to be tested again. Must be ordered separately.
11. Timer
12. Vortex mixer

Reagents Provided

1. **Microplate** - Ready to use
   Contents: One 96-well (12x8) anti-aldosterone polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.

2. **HRP Conjugate** - Ready to use
   Contents: Aldosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.

3. **Aldosterone Calibrators** – A – F Ready to Use
   Contents: Six bottles containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantites of aldosterone. Calibrator concentrations*: 0, 15, 50, 200, 500 and 1000 pg/mL.
   * Approximate values - please refer to vial labels for exact concentrations.
   Volume: Calibrators A–F: 1 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.

4. **Aldosterone Controls** - Ready to Use
   Contents: Two bottles containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of aldosterone. Refer to the QC certificate for the target values and acceptable ranges.

Volume: 1 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for 4 weeks.

5. **Wash Buffer Concentrate** - Requires Preparation (X10)
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.
   After Preparation: 2 weeks assuming good laboratory practices are followed. To prevent microbial growth, prepare in a clean container and store at 2-8°C when not in use.
   Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used, dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **TMB Substrate** - Ready to Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in A non-DMF or DMSO-containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.

7. **Stop Solution**- Ready to Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label.
   After Opening: Stable for 4 weeks.
   Safety:  Warning. Refer to product SDS.

Assay Procedure

All reagents must reach room temperature before use. Calibrators, controls, and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare 1X working wash buffer.
3. If serum or plasma samples are being used there is no sample preparation required. If urine samples are being used, they must be diluted prior to use (see Sample Pretreatment section).
4. Remove the required number of strips from the microplate and assemble into a plate frame. Return any unused strips to the bag with desiccant, re-seal, and store in the refrigerator.

5. Pipette 50 µL of each calibrator, contro,l and sample (serum, plasma or diluted urine) into assigned wells in duplicate.
6. Pipette 100 µL of the aldosterone-HRP conjugate into each well (the use of a multichannel pipette is recommended).
7. Incubate on a plate shaker (~180 oscillations / minute on a reciprocating shaker or ~600 rpm on an orbital shaker) for 60 minutes at room temperature.
8. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Perform a 3-cycle wash using 350 µL/well of 1X working wash buffer (3 x 350µL). One cycle consists of aspirating all wells then filling each well with 350 µL/well of 1X working wash buffer. After the final wash cycle, aspirate all wells then tap the plate firmly against absorbent paper to remove any residual liquid.

   Manual: Perform a 3-cycle wash using 350 µL/well of 1X working wash buffer (3 x 350µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of 1X working wash buffer into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents of the wells over a waste container. Tap the plate firmly against absorbent paper to remove any residual liquid.

9. Pipette 150 µL of the TMB substrate into each well at timed intervals (the use of a multi-channel pipette is recommended).
10. Incubate on a plate shaker (~180 oscillations / minute on a reciprocating shaker or ~600 rpm on an orbital shaker) for 20 minutes at room temperature.
11. Pipette 50 µL of stop solution into each well at the same timed intervals as the TMB substrate (the use of a multi-channel pipette is recommended). Gently tap the microplate frame to mix the contents of the wells.
12. Measure the optical density (absorbance) using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the stop solution.

Calculations

1. Calculate the mean optical density of each calibrator, control, and sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and samples using the mean optical density values and the calibrator curve.
4. If a serum or plasma sample reads greater than 1000 pg/mL then dilute it with Aldosterone Serum / Plasma Diluent (available separately) at a dilution of no more than 1:8. The result obtained must be multiplied by the dilution factor.
5. If a urine sample reads more than 1000 pg/mL then dilute it with Aldosterone Urine Diluent (available separately) at a dilution of no more than 1:8 (from the original 1:50 dilution). The result obtained must be multiplied by the dilution factor.

Urine Sample Calculations

The final concentration of the urine samples must consider the 1:50 dilution that was performed during the sample pre-treatment step and the total volume of collected 24-hour urine.

Calculate the final urine sample Aldosterone concentration using the following formula:

Final urine sample Aldosterone concentration (pg/24-hour) =
Concentration calculated from calibrator curve x **50** (dilution factor) x **Volume of 24-hour urine (in mL)**.

To obtain a value in µg/24-hour (h), divide the pg/24-hour value by 1 x 10^6 (1,000,000).

Example:
A pre-treated urine sample was tested with the Aldosterone ELISA kit, and the obtained concentration was 100 pg/mL.
The volume of the collected 24-hour urine was 1200 mL.

The aldosterone concentration of the urine sample is:
(100 pg/mL x 50 x 1200 mL) ÷ 1,000,000 = 6 µg/24 h.

Note: Do not perform any calculations to samples that did not undergo the sample pre-treatment (dilution) step (e.g. kit controls).

Quality Control

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

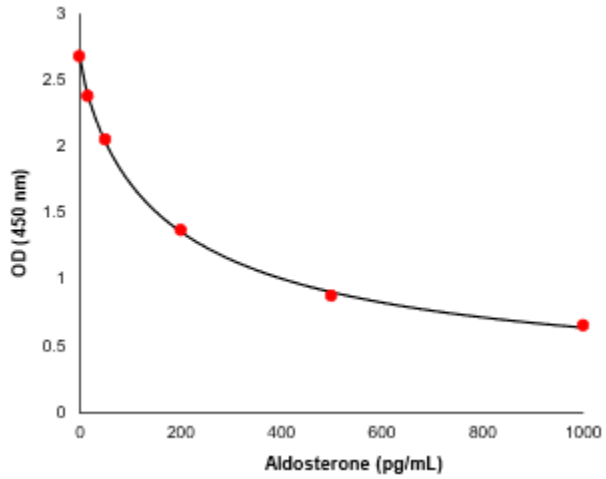
Typical Tabulated Data

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.680	100	0
B	2.382	89	15
C	2.053	77	50
D	1.374	51	200
E	0.884	33	500
F	0.657	25	1000
Unknown	1.705	-	104.1

Typical Calibrator Curve

Sample curve only. **Do not** use to calculate results.



General Information

In the case of product complaints, the user shall submit in writing to ALPCO a description of the complaint and provide accompanying data and/or information.

ALPCO guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

ALPCO liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.