Aldosterone ELISA Assay Kit

Catalog Number:
ALD31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.0 (18 JUN 24)

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INTENDED USE

The Eagle Biosciences Aldosterone ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Aldosterone in human serum, plasma and urine by an enzyme immunoassay. The Eagle Biosciences Aldosterone ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Aldosterone is a potent mineralocorticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the reabsorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension.

Measurement of aldosterone levels in serum in conjunction with plasma renin activity levels 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 summary, the precise and accurate measurement of aldosterone by enzyme immunoassay can be an important addition to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate wells. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured with a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of aldosterone in the sample. A set of standards is used to plot a standard curve from which the amount of aldosterone in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.

d. Wash hands thoroughly after performing the test.
e. 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 expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
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 to all customers to prepare their own control materials or serum 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 their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit should be reported as > 1000 ng/ml and must not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the 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 stopping 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 absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used 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 wells, 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 wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. This kit is intended for research use only and should not be used as a diagnostic tool.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.
All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Serum: Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Plasma: Approximately 0.2 mL of plasma is required per duplicate determination. Collect 4–5 mL of blood into EDTA plasma tubes. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Urine: 24-hour urine into a specimen collection container. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

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

Urine: Dilute urine samples 1:50 in urine diluent right before the test. Do not stored diluted urine samples.

1. Pipette 0.98 mL of the Urine Diluent into a new polypropylene microcentrifuge tube
2. Pipette 20 µL of the urine specimen into the tube from step 1 that contains 0,98 mL of urine diluent
3. Close the tube and label it with specimen identification information
4. Mix the contents of the tube by vortexing

Note* Different volumes of Urine Diluent and urine speciomen may be used provided that the required 1:50 ratio is maintained.

Pre-treated urine specimens must be assayed on the same day as they were prapred. Do not store pre-treated urine specimens beyond this time limit.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 100, 150, 350 and 0.98 µL (for urine samples only)
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)
6. Urine Diluent – Required for the dilution of urine specimens before assaying.
7. Serum and Plasma Diluent – Required if high samples (>1000 pg/mL) are to be tested again.

REAGENTS PROVIDED

1. **Anti-Aldosterone Polyclonal Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   - Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **Aldosterone-Horseradish Peroxidase (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: 12 months or as indicated on label.

3. **Aldosterone Calibrators** — Ready To Use
   - Contents: Six vials containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of aldosterone.

   Calibrator concentrations*: 0, 15, 50, 200, 500 and 1000 pg/mL
   * Approximate value – please refer to vial labels for exact concentrations.

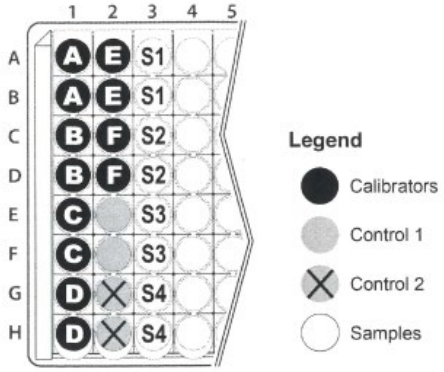
Volume: Calibrators A-F: 1 mL/vial
Storage: Refrigerate at 2–8°C.
Stability: 12 months in unopened vials or as indicated on label.

4. **Aldosterone Controls** — Ready to Use
   Contents: Two vials containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of aldosterone. Refer to vial labels for the acceptable range.
   Volume: 1 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label.

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: 12 months or as indicated on label.
   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 buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

7. **Stopping Solution** — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen 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. 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 Specimen Preparation section). Prepare the working wash buffer (see wash buffer concentrate under the Reagents Provided section).
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette **50 µL** of each calibrator, control and specimen sample (serum, plasma or diluted urine) into correspondingly labelled wells in duplicate.
4. Pipette **100 µL** of the aldosterone-HRP conjugate into each well (the use of a multichannel pipette is recommended).
5. **Incubate** on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 60 minutes at room temperature.
6. **Wash** the wells 3 times each time with 350 µL/well of working wash buffer solution. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
7. Pipette **150 µL** of the TMB substrate into each well at timed intervals (the use of a multichannel pipette is recommended).
8. **Incubate** on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 20 minutes at room temperature or until calibrator A attains dark blue colour for desired OD).
9. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 7. Mix thoroughly by gently tapping the plate.
10. **Measure** the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the serum and plasma samples directly off the calibrator curve.
5. Read the values of the urine samples directly off the curve and multiply by a factor of 50. Next, multiply by the volume of collected 24-hour urine (in mL) to obtain values in pg/24 hour. Finally, divide the pg/24-hour values by $1x10^6$ (1,000,000) to obtain values in µg/24 hour.
6. If a serum or plasma sample reads greater than 1000 pg/mL then dilute it with the Serum and Plasma Diluent at a dilution of no more than 1:8. The result obtained must be multiplied

by the dilution factor. If a urine sample reads more than 1000 pg/mL then dilute it with the urine diluent 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 Specimen Calculations

The final concentration of the urine specimen samples must take into account the 1:50 dilution that was performed during the specimen pre-treatment step and th total volume of collected 24-hour urine.

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

Final urine specimen 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 vlue in µg/24-hour (h), divide the pg/24-hour value by 1 x 10^6 (1,000,000).

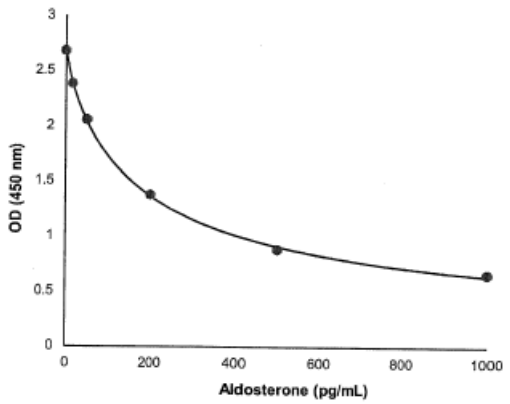
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Aldosterone (pg/mL)
A	2.680	100	0
B	2.382	89	15
C	2.053	77	50
D	1.375	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.



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Warranty Information

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.