



EAGLE
BIOSCIENCES

Aldosterone ELISA Kit

Catalog Number:

ALD31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 6.0 (effective 06NOV23)

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INTENDED USE

The Eagle Biosciences Aldosterone ELISA Kit (enzyme-linked immunoassay kit) is intended for the quantitative measurement of aldosterone in human serum. The Eagle Biosciences Aldosterone ELISA Kit is for research use only and not to be used in diagnostic procedures.

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

LIMITATIONS RELATED TO INTENDED USE

1. This kit is intended for research use only and is not to be used for any diagnostic procedures.

PRINCIPLE OF THE ASSAY

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

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens 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.
- 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.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- 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.
- 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.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this 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 reagent storage.

- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate 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 should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- 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.
- 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.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- 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 shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- 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 participant is established.
- 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.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. 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 direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

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

Urine: Approximately 0.05 mL of urine is required per duplicate determination. Collect urine into a specimen 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 specimens (refer to *Calculations*). Urine samples may be stored at 2-8°C for up to 7 days.

Consider all human specimens as possible biohazards materials and take appropriate precautions when handling.

Specimen Pre-Treatment

Serum and Plasma: Specimen pre-treatment is not required.

Urine: All urine specimens must be diluted 1:50 in the Urine Diluent before being used in the test. Follow the specimen pre-treatment procedure as stated below for each specimen that is to be tested:

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

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

Consider all human specimens as possible biohazardous materials.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Calibrated single-channel pipette to dispense 20 µL (for urine samples only), 50 µL, and 0.98 mL (for urine samples only).
- Calibrated multi-channel pipettes to dispense 50 µL, 100 µL, and 150 µL.
- Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
- Automatic microplate washer (recommended)
- Microplate shaker:
 - Orbital shaker (3 mm diameter) set to 600 rpm or

- Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
- Disposable pipette tips
- Distilled or deionized water
- Calibrated absorbance microplate reader with a 450 nm and an upper OD limit of 3.0 or greater
- Polypropylene tubes for sample pre-treatment (for urine samples only).
- Aldosterone Serum/Plasma Diluent – only required if it is necessary to dilute serum or plasma samples > 1000 pg/mL. Must be ordered separately.
- Aldosterone Urine Diluent – only required if urine specimens will be tested. Must be ordered separately.
- Vortex mixer.

REAGENTS PROVIDED

1. Microplate

Contents: One anti-aldosterone polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.

Format: Ready to Use

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. HRP-Conjugate

Contents: One bottle containing aldosterone-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.

Format: Ready to Use

Volume: 15 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

3. Standard A - F

Contents: Six bottles of standard containing specified aldosterone concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of aldosterone.

Listed below are approximate concentration, please refer to vial labels for exact concentrations.

Concentrations: 0, 15, 50, 200, 500, 1000 pg/mL

Format: Ready to Use

Volume: 1.0 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

4. Control 1 - 2

Contents: Two bottles of control containing different aldosterone concentrations. 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.

Format: Ready to Use
Volume: 1.0 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

5. TMB Substrate

Contents: One bottle containing a tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Format: Ready to Use
Volume: 16 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks.

6. Stopping Solution

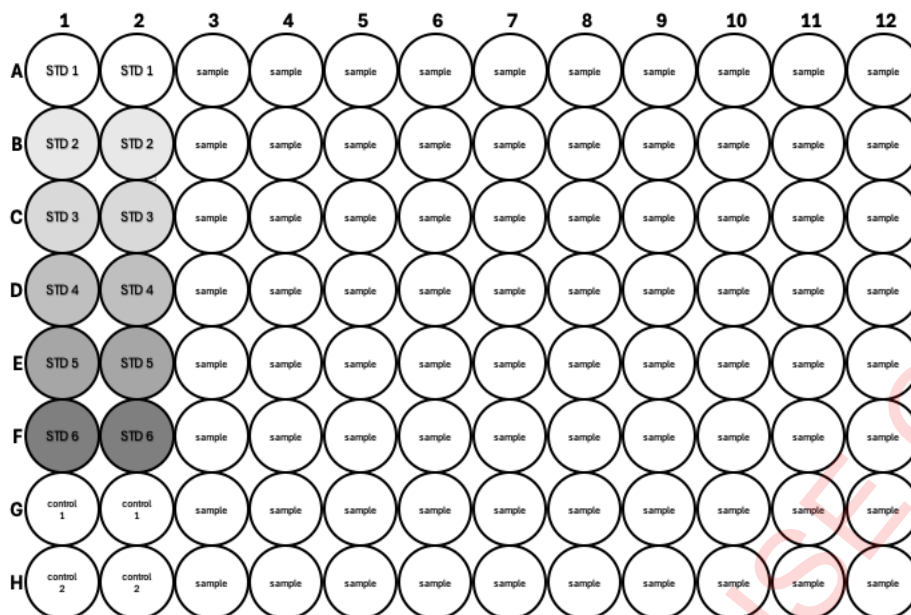
Contents: One bottle containing 1M sulfuric acid.
Format: Ready to Use
Volume: 6 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks

7. Wash Buffer Concentrate (10x)

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative
Format: Concentrated; Requires Preparation
Volume: 50 mL/ bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation: **Dilute 1:10** in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT*



* Layout subject to change based on standard and control quantities

ASSAY PROCEDURE

Specimen Pre-Treatment: All urine samples that will be tested must be pre-treated before being tested. Do not pre-treat the standards and kit controls as they are provided and ready to use.

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, 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.
2. Prepare the Wash Buffer Working Solution (see Reagents Provided).
3. If urine samples are being tested, they must be pre-treated prior to use. Refer to section *Specimen Pre-Treatment & Storage*.
4. Plan the microplate wells to be used for standards, controls, and samples. (See *Recommended Assay Layout*). Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
5. **Pipette 50 μ L** of each standard, control and specimen sample into assigned wells.
6. **Pipette 100 μ L** of the HRP Conjugate Working solution into each well (the use of a multichannel pipette is recommended.)
7. **Incubate** the microplate inside on a microplate shaker** for **60 minutes** at room temperature.
8. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
 - a. Automatic: Using an automatic microplate washer, perform a 3-cycle wash using **350 μ L/well** of Wash Buffer Working Solution (3 x 350 μ L). One cycle consists of aspirating all wells then filling each well with 350 μ L of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.
 - b. Manually: Using an automatic microplate washer, perform a 3-cycle wash using **350 μ L/well** of Wash Buffer Working Solution (3 x 350 μ L). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 μ L of Wash Buffer Working Solution into each well using a multi-channel pipette. After

the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

9. **Pipette 150 µL** of TMB substrate into each well (the use of a multi-channel pipette is recommended).
10. **Incubate** the microplate on a microplate shaker** for **20 minutes** at room temperature.
11. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

**See section *Reagents and Equipment Needed But Not Provided* for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density of each standard, control, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a standard curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the standard curve.
4. If a serum or plasma sample reads more than 1000 pg/mL and needs to be diluted and retested, then dilute with Aldosterone Serum/Plasma Diluent (see section *Reagents and Equipment Needed But Not Provided*) not 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 and needs to be diluted and retested, then dilute the 1:50 diluted sample with the Aldosterone Urine Diluent (see section *Reagents and Equipment Needed But Not Provided*) not more than 1:8. 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 the 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 standard 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 1×10^6 (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:

$(100 \text{ pg/mL} \times 50 \times 1200 \text{ mL}) / 1,000,000 = 6 \text{ µg/24 h.}$

Do not perform any calculation to samples that did not undergo the specimen 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 standard A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The standard with the highest concentration meets the % binding acceptable ranges as stated in the QC Certificate. % Binding = (OD of standard/OD of standard 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.

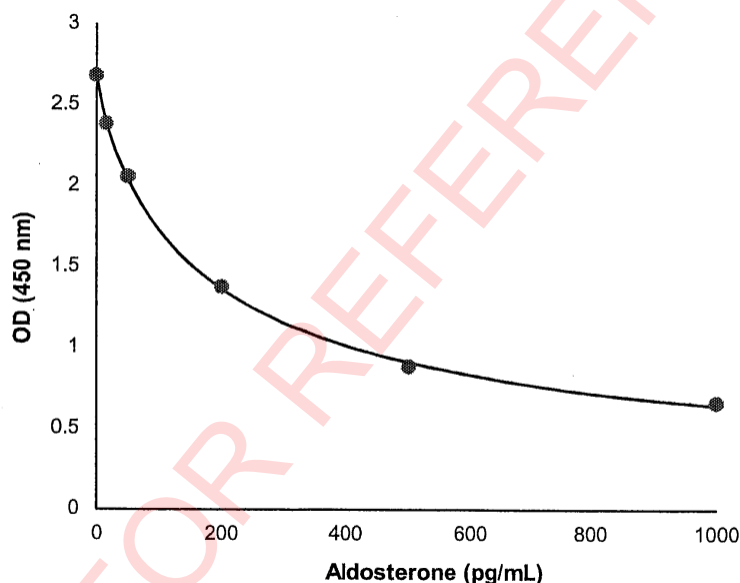
TYPICAL DATA

Sample data only. Do not use to calculate results.

Standard	Mean OD (450 nm)	% Binding	Value (ng/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 STANDARD CURVE

Sample curve only. Do not use to calculate results.



WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new



Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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