



Manufactured for:  
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**Direct ELISA Kit**  
**ALDOSTERONE**

Cat. No.: IB59101

Version: 6.0

Effective: October 25, 2010

**INTENDED USE**

For the direct 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 TEST**

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in calibrators, control and unknowns being tested) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. 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 on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of aldosterone in the unknown. A set of calibrators is used to plot a calibration curve from which the amount of aldosterone in unknowns and controls can be directly read.

**PROCEDURAL CAUTIONS AND WARNINGS**

1. 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.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and unknowns.
5. All kit reagents and unknowns should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and unknowns.
6. A calibrator curve must be established for every run.
7. The control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) 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.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, unknown, calibrator and control.

13. 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.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

**LIMITATIONS**

1. All the reagents within the kit are calibrated for the direct determination of aldosterone in human serum, plasma and urine. The kit is not calibrated for the determination of aldosterone in saliva or other types of human or animal unknowns.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum or plasma.
3. Any unknowns or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to inaccurate results.
4. Only calibrator A may be used to dilute any elevated unknowns. Only the urine diluent may be used to dilute any high urine unknowns. The use of any other reagents may lead to inaccurate results.
5. The occurrence of heterophilic antibodies in subjects regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. The subject's background, including the frequency of exposure to animals/products, should be considered if false results are suspected.

**SAFETY CAUTIONS AND WARNINGS**  
**POTENTIAL BIOHAZARDOUS MATERIAL**

Human serum that may be used in the preparation of the calibrators and control has been tested and found to be non-reactive 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. However no test method 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 biohazardous material.

**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.

**COLLECTION AND HANDLING OF UNKNOWNNS**

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: Approximately 0.2 ml of urine is required per duplicate determination. Collect 24-hour urine into a 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 unknowns as possible biohazardous materials and take appropriate precautions when handling.

**PRETREATMENT OF UNKNOWNNS**

Serum and plasma: This assay is a direct system; no pretreatment is necessary.

Urine: Dilute urine 1:50 in urine diluent before use.

Example: To 1 ml of urine diluent, add 20 µl of urine unknown being tested.

**REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Precision pipettes to dispense 50, 100, 150 and 300 µl
  2. Disposable pipette tips
  3. Distilled or deionized water
  4. Plate shaker
  5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater\* (see assay procedure step 10).
  6. Urine Diluent - Required if urine unknowns are to be analysed. Used for dilution of urine unknowns before assaying.
- Cat. No.: CAN-ALD-450-11

**REAGENTS PROVIDED**

**1. Rabbit Anti-Aldosterone Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.**

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.

**2. Aldosterone-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.**

Contents: Aldosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.  
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12ml of assay buffer. Discard any that is left over.

**3. Aldosterone Calibrators - Ready To Use.**

Contents: Six vials containing aldosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of aldosterone.

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

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/ml	2.0 ml
Calibrator B	15 pg/ml	0.6 ml
Calibrator C	50 pg/ml	0.6 ml
Calibrator D	200 pg/ml	0.6 ml
Calibrator E	500 pg/ml	0.6 ml
Calibrator F	1000 pg/ml	0.6 ml

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the calibrators should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

**4. Controls - Ready To Use.**

Contents: Two vials containing aldosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of aldosterone. Refer to vial labels for expected value and acceptable range.

Volume: 0.6 ml/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

**5. Wash Buffer Concentrate - Requires Preparation.**

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 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

**6. Assay Buffer - Ready To Use.**

Contents: One vial containing a protein-based buffer with a non-mercury preservative.  
Volume: 15 ml/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.

**7. 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: 12 months or as indicated on label.

**8. Stopping Solution - Ready To Use.**

Contents: One vial containing 1M sulfuric acid.  
Volume: 6 ml/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.

**ASSAY PROCEDURE**

Pretreatment of Unknowns:

**Serum and plasma: None.**

**Urine: Dilute 1:50 in Urine Diluent Before Use.**

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

1. Prepare working solutions of the aldosterone-HRP conjugate and wash buffer. Dilute any urine unknowns if they are to be analyzed.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µl of each calibrator, control and unknown (serum or diluted urine) into correspondingly labelled wells in duplicate.
4. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 µl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 15-20 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.
10. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

\* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of the unknowns being tested.

**RESULTS**

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 curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the serum and plasma unknowns directly off the calibrator curve.
5. Read the values of the urine unknowns 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  $1 \times 10^6$  to obtain values in µg/24 hour.
6. If a serum or plasma reads more than 1000 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor. If a urine unknown reads more than 1000 pg/ml then dilute it with the urine diluent at a dilution of no more than 1:2 (from the original 1:50 dilution). The result obtained should be multiplied by the dilution factor.

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