DRG® Aldosterone ELISA (EIA-4128)

Revised 7 Apr. 2011 rm (Vers. 7.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

For determination of Aldosterone in human serum, plasma and urine by an enzyme immunoassay .

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 samples) 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 sample. A set of calibrators is used to plot a standard curve from which the amount of aldosterone in samples 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 human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
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, sample, standard 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 specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum, or plasma.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only Calibrator A may be used to dilute any high samples.
   Only the urine diluent may be used to dilute any high urine samples.
   The use of any other reagents may lead to false results.

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 blood specimen.

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:

Approximately 0.2 ml of urine is required per duplicate determination. Collect 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: This assay is a direct system; no specimen pretreatment is necessary.

Urine: Dilute urine samples 1:50 in urine diluent before use.
Example: To 1 ml of urine diluent, add 20 µl of urine sample.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

Precision pipettes to dispense 50, 100, 150 and 300 µl

1. Disposable pipette tips
2. Distilled or deionized water
3. Plate shaker
4. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).
5. Urine Diluent - Required if urine samples are to be analysed. Used for dilution of urine specimens before assaying. Available in any quantity.

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 –50X-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.

Standard	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 label 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 control should be used within 14 days or aliquoted and stored frozen.
Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate**
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. **Stop 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

Specimen Pretreatment:

Serum and Plasma: None.

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

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. Prepare working solutions of the aldosterone-HRP conjugate and wash buffer. Dilute any urine samples 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 standard, control and specimen sample (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 Standard 0 attains dark blue colour for desired OD).
9. Pipette 50 µl of stop 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 specimen/control samples.

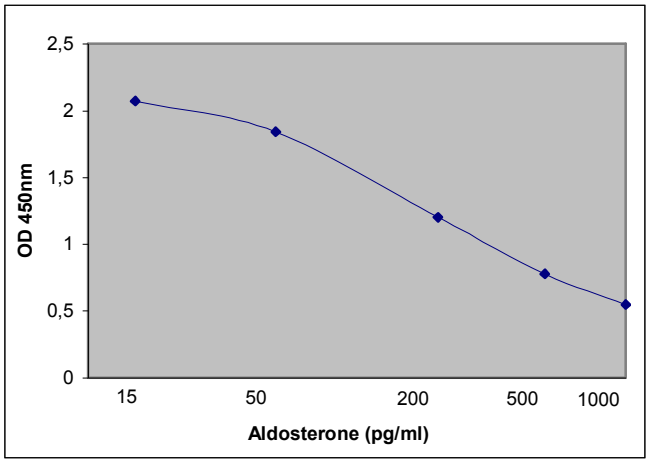
CALCULATIONS

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 Standard 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 samples directly off the Standard 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 $1x10^6$ to obtain values in µg/24 hour.
6. If a serum or plasma sample reads more than 1000 pg/ml then dilute it with Standard 0 at a dilution of no more than 1:8. The result obtained should 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 1:2 (from the original 1:50 dilution). The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (pg/ml)
A	2.267	2.197	2.232	0
B	2.102	2.037	2.070	15
C	1.848	1.836	1.842	50
D	1.210	1.190	1.200	200
E	0.763	0.788	0.776	500
F	0.542	0.550	0.549	1000
Unknown	1.522	1.493	1.508	112

TYPICAL CALIBRATOR CURVE



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

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Version 10-25-10~rm