Human Aldosterone ELISA Kit

Cat. No.: DEIA2002
Pkg. Size: 96T

Intended use
For determination of Aldosterone by enzyme immunoassay in human serum by enzyme immunoassay. Hydrolysis is necessary for the determination of Aldosterone in urine.

General Description
Aldosterone is a potent mineral corticoid 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 levels can be used to differentiate between primary and secondary aldosteronism. The measurement of aldosterone in concert with selective suppression and stimulation tests can be used to further differentiate primary aldosteronism into two basic types:
1. Primary aldosteronism caused by an adenoma of one or both adrenals.
2. Primary aldosteronism caused by adrenal hyperplasia.

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 standards, controls 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 standards is used to plot a standard curve from which the amount of aldosterone in samples and controls can be directly read.

Reagents And Materials 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.
   Contents: Aldosterone-biotin and avidin-HRP conjugates 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 the aldosterone-biotin:avidin-HRP concentrate 1:50 in assay buffer before use. 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 protein-based buffer with a non-mercury preservative. Prepared by spiking matrix...
with a defined quantity of aldosterone.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.
Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards 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 protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of aldosterone.
Refer to vial labels for the acceptable range.
Volume: 0.5 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 – 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 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°C to 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.

**Materials Required But Not Supplied**
1. Precision pipettes to dispense 50, 100, 150 and 300 μl
2. Disposable pipette tips
3. Distilled or deionized water
4. 3.2 N HCl and 3.2 N NaOH (for urine analysis)
5. Glass or polypropylene tubes (for urine analysis)
6. Water bath (for urine analysis)
7. Plate shaker
8. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay step 10)
Specimen Collection And Handling

**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.

**Urine:**
Approximately 1 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.

**Serum Pretreatment**
No specimen pretreatment is necessary.

**Urine Pretreatment**
1. Label one glass or polypropylene tube for each urine sample.
2. Pipet 1 mL of each urine sample into an appropriate tube.
   * If the sample is cloudy, first centrifuge the urine and work with the supernatant.
3. Hydrolysis: Add 0.1 mL of 3.2 N HCl (not supplied) to every tube. Cap securely and heat for 1 hour at 60°C in the dark.
   * 3.2 N HCl can be made by adding 1 mL of concentrated HCl (12 N) to 2.75 mL distilled water.
4. Neutralization: Add 0.1 mL of 3.2 N NaOH to every tube and mix gently and thoroughly.
   * 3.2 N NaOH can be made by dissolving 1.28 grams of NaOH pellets into 10 mL distilled water.

**Assay Steps**

**Specimen Pretreatment:**
Serum: None.
Urine: Hydrolysis, Neutralization and Dilution (see detailed instructions under Urine Pretreatment)
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 conjugate and wash buffer.
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 samples (serum or treated 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 10-15 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 control samples.

**Calculation**
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 samples directly off the calibrator curve. If a serum sample reads more than 2000 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.
5. Read the values of the urine samples directly off the curve and multiply by a factor of 60 (the original urine samples are diluted 1-in-1.2 and 1-in-50, see the urine pretreatment). Next, multiply by the volume of collected 24-hour urine (in litres). Finally, divide this figure by 1000 to obtain values in μg/24 hour. If a urine sample reads more than 2000 pg/ml then dilute it with the calibrator A 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 Standard Curve**

The following data were obtained for the various standards over the range of 0 to 1400 pg/mL Rat IFN-γ.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.724</td>
<td>2.569</td>
<td>2.647</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2.455</td>
<td>2.499</td>
<td>2.477</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>2.115</td>
<td>2.103</td>
<td>2.109</td>
<td>80</td>
</tr>
<tr>
<td>D</td>
<td>1.351</td>
<td>1.401</td>
<td>1.376</td>
<td>300</td>
</tr>
<tr>
<td>E</td>
<td>0.837</td>
<td>0.810</td>
<td>0.824</td>
<td>800</td>
</tr>
<tr>
<td>F</td>
<td>0.528</td>
<td>0.521</td>
<td>0.525</td>
<td>2000</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.885</td>
<td>1.805</td>
<td>1.845</td>
<td>138</td>
</tr>
</tbody>
</table>
Precautions

1. Human serum that may be used in the preparation of the standards and controls 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.

2. 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.

Limitations

1. All the reagents within the kit are calibrated for the direct determination of aldosterone in human serum and urine. The kit is not calibrated for the determination of aldosterone in other specimens of human or animal origin.

2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.

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 urine and any high serum samples. The use of any other reagents may lead to false results.

REFERENCES


