INTENDED USE
Competitive immunoenzymatic colorimetric method for quantitative determination of Aldosterone concentration in human serum, human plasma or urine.

The Aldosterone kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE
Aldosterone is a steroid hormone produced by the adrenal cortex in the adrenal gland, is the most potent mineralocorticoid in humans, it regulate sodium and potassium balance in the blood.

Aldosterone secretion appears to be stimulated primarily through the renin-angiotensin system. Acting on mineralocorticoid receptors (MR) on principal cells in the collecting ducts of the kidneys, it increases the permeability of their apical (luminal) membrane to potassium and sodium and activates their basolateral Na+/K+ pumps, stimulating ATP hydrolysis, reabsorbing sodium (Na+) ions and water into the blood, and excreting potassium (K+) ions into the urine. Aldosterone regulate plasma bicarbonate (HCO₃⁻) levels and its acid/base balance.

Aldosterone is responsible for the reabsorption of about 2% of filtered sodium in the kidneys. Plasma aldosterone levels normally vary with body position (upright>supine) and salt intake. Overall plasma aldosterone levels show a circadian rhythm which is similar to but less marked than cortisol, with peak levels in the early morning; about 75% of the daily production is secreted between 04:00 am and 10:00 am each day. Age-related levels tend to decline from fetal through adult life.

Abnormally high plasma aldosterone concentrations can occur in adenomas, glucocorticoid-responsive hyperaldosteronism, idiopathic.

Abnormally low aldosterone secretion occurs in a number of conditions including salt-wasting forms of congenital adrenal hyperplasia, nephropathy, and renal tubular acidosis.

2. PRINCIPLE
The principle of the following enzyme immunoassay test follow the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding siters on the microwell plate. The washing step 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 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.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit
1. Aldosterone Standards (6x1 vial = 1 mL)
STD0 REF DCE002/5306-0
STD1 REF DCE002/5307-0
STD2 REF DCE002/5308-0
STD3 REF DCE002/5309-0
STD4 REF DCE002/5310-0
STD5 REF DCE002/5311-0

2. Aldosterone Conjugate (1 vial) 0.3 mL
Aldosterone-HRP conjugate REF DCE002/5302-0

3. Coated Microplate (1 microplate breakable coated with anti-Aldosterone)
REF DCE002/5303-0

4. Incubation Buffer (1 vial) 30 mL
Phosphate buffer 50 mM pH 7.5; BSA 1 g/L; stabilizer REF DCE001-0

5. 50X Conc. Wash Solution (1 vial) 20mL
NaCl 45 g/L; Tween-20 55 g/L REF DCE006/5306-0

6. TMB-substrate (1 bottle) 12 mL
H₂O₂; TMB 0.26 g/L (avoid any skin contact) REF DCE004-0

7. Stop solution (1 bottle) 12 mL
Sulfuric acid 0.15 mol/L (avoid any skin contact) REF DCE005-0
3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader (450 nm)

\section*{Note}
Store all reagents between 2-8°C in the dark.
Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.

4. PRECAUTIONS
\begin{itemize}
\item The reagent contain Proclin 300\textsuperscript{R} as preservative.
\item Avoid the exposure of reagent TMB/H\textsubscript{2}O\textsubscript{2} to directed sunlight, metals or oxidants.
\item Maximum precision is required for reconstitution and dispensation of the reagents.
\item Do not use different lots of reagents.
\item Do not use heavily haemolized samples.
\item This method allows the determination of Aldosterone from 20 to 2000 pg/mL
\item The treatment with natural or synthetic steroids can affect blood levels of aldosterone.
\end{itemize}

5. PROCEDURE

5.1. Preparation of the Standard
\begin{itemize}
\item The standard has approx. the following concentration:
\end{itemize}

\begin{tabular}{|c|c|c|c|c|c|}
\hline
\text{pg/mL} & S_{0} & S_{1} & S_{2} & S_{3} & S_{5} \\
\hline
0 & 20 & 80 & 300 & 800 & 2000 \\
\hline
\end{tabular}

Once open are stable six months at +4°C.

5.2. Preparation of Wash Buffer
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

5.3. Preparation of Diluted Conjugate
Prepare immediately before use.
Dilute the Aldosterone-Conjugate 1:5 into Incubation buffer (e.g. 20 μL of Conjugate can be diluted to 1 mL with Incubation buffer). Mix gently for almost ten minutes.

5.4. Preparation of the Sample
The determination of Aldosterone can be performed in human serum, human plasma or in urine. Store the sample at -20°C if the determination is not performed on the same day of the sample connection.
For sample with concentration over 2000 pg/mL dilute the sample with zero Standard.
For Urine determination please see annex A.

5.5. Procedure
As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_{0}-S_{5}), and for each sample, one for Blank.

| Pipette: |
| --- | --- | --- |
| Sample | Standard | Sample | Blank |
| Sample | --- | 50 μL | --- |
| Standards S_{5} | 100 μL | --- | --- |
| Diluted Conjugate | 100 μL | 100 μL | --- |

Mix well
Incubate at 37°C for 1 hour
Remove the contents from each well and wash the wells with 300 μL of diluted Wash Solution. Repeat the washing procedure two more times by draining the wash completely, for a total number of three washing.

| Pipette: |
| --- | --- | --- |
| TMB-Substrate | Standard | Sample | Blank |
| Stop solution | 100 μL | 100 μL | 100 μL |

Shake the microplate gently.
Read the absorbance (E) at 450 nm against Blank.

6. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of Aldosterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

7. LIMITATIONS OF PROCEDURE

7.1. Assay Performance
Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or
decantation wash step(s) may result in poor replication and spurious results.

7.2. Results interpretation
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

8. RESULTS

8.1. Mean Absorbance
Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample

8.2. Standard Curve
Plot the values of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of Results
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

9. REFERENCE VALUES

<table>
<thead>
<tr>
<th>Serum:</th>
<th>pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Adult</td>
<td>Mean</td>
</tr>
<tr>
<td>Early Morning, Supine</td>
<td>68.9</td>
</tr>
<tr>
<td>Upright, 2 Hours</td>
<td>109.2</td>
</tr>
<tr>
<td>24-Hour Urine:</td>
<td>µg/day</td>
</tr>
<tr>
<td>Normal Adult</td>
<td>Volume</td>
</tr>
<tr>
<td>Urine</td>
<td>1650 mL</td>
</tr>
</tbody>
</table>

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision
10.1.1. Intra Assay Variation
Within run variation was determined by replicate measurements (16x) of two different control sera in one assay. The within assay variability is ≤ 9.7%.

10.1.2. Inter Assay Variation
Between run variation was determined by replicate measurements (10x) of three different control sera in different lots of kits. The between assay variability is ≤ 11%.

10.2. Accuracy
The recovery of 0; 300; 800; 2000 (pg/mL) added to two patient samples gave an average value (±SE) of 103.94% ± 2.78%.

10.3. Sensitivity
The lowest detectable concentration of Aldosterone that can be distinguished from the zero standard is 7 pg/mL at the 95% confidence limit.

10.4. Specificity
The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>100.0%</td>
</tr>
<tr>
<td>11-Deoxycorticosterone</td>
<td>1.10%</td>
</tr>
<tr>
<td>Androstened</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Cortisone</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>11-Deoxycorticisol</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>21-Deoxycorticisol</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Estradiol</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Estriol</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt; 0.001%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt; 0.001%</td>
</tr>
</tbody>
</table>

10.5. Correlation with RIA
The Dia.metra Aldosterone ELISA was compared to a commercially available Aldosterone RIA assay.

56 serum samples were tested.
The linear regression curve was calculated
\[ y = 1.03 x + 1.64 \]
\[ r^2 = 0.99 \]

\( y \) = Aldosterone Diametra Elisa Kit
\( x \) = Aldosterone Adaltis MAIA RIA Kit

14 urine samples were tested.
The linear regression curve was calculated
\[ y = 0.86 x + 12.53 \]
\[ r^2 = 0.92 \]

\( y \) = Aldosterone Diametra Elisa Kit
\( x \) = Aldosterone Adaltis MAIA RIA Kit

11. WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

DiaMetra S.r.l. Headquater: Via Garibaldi, 18 – 20090 SEGRATE (MI) Tel. 0039-02-2139184 – 02-26921595 – Fax 0039–02–2133354.
Manufact: Via Giustozzi, 35/35a (già via Bartolomei) – Z.I Paciana – 06034 FOLIGNO (PG) ITALY. Tel. 0039-0742–24851 Fax 0039–0742–316197 E-mail: info@diametra.com
Annex A

Sample Preparation: Urine

Precautions: Ethyl acetate is a volatile, flammable organic solvent. Conduct the evaporation step under a fume hood equipped with an explosion-proof exhaust fan. Avoid open flames, and do not pipet by mouth. The ethyl acetate must be of at least spectrophotometric grade.

1. Label one glass or polypropylene tube for each urine sample. The tubes should have tight-fitting caps and be able to withstand centrifuging at 1500xg.

2. Pipet 250 µL of each urine sample into the appropriate tube. If the sample is cloudy or if a precipitate has formed, first centrifuge the urine and work with the supernatant.

3. **Hydrolysis:** Add 25 µL of 3.2 N HCl (not supplied) to every tube. Cap securely and incubate for 24 hours at room temperature in the dark. 3.2 N HCl can be made by adding 1.0 mL concentrated HCl (12 N) to 2.75 mL distilled water. Do not add water to concentrated acid, since this may cause splattering.

4. **Extraction:** Add 2.5 mL ethyl acetate (not supplied) to every tube. Cap securely.

5. Mix by gentle inversion for 60 minutes. Use a mechanical rotator set at 15–20 revolutions-per-minute.

6. Centrifuge for 5 minutes at about 1500xg, to separate the two layers. Any sample partially emulsified should be shaken vigorously and centrifuged again.

7. **Evaporation:** Transfer exactly 100 µL of the upper (ethyl acetate) phase cleanly into one plain (uncoated) 12x75 mm polypropylene tube. (Do not use polystyrene) Pipet directly to the bottom of the tube using a positive-displacement micropipet. The remainder of the ethyl acetate phase may be retained for future use simply by freezing the extraction tube at –20°C; it is not necessary to separate the ethyl acetate phase from the aqueous phase.

8. Evaporate to complete dryness under a gentle stream of nitrogen at 37°C.

9. Add 0.5 mL of the Incubation buffer (auxiliary reagent) or saline solution NaCl 0.9%. Thoroughly resuspend the extract by vortexing.

10. Transfer 50 µL of Resuspend to the well of coated microplate

Proceed with the assay procedure, as described in the IFU (using the resuspended as normal sample)

**Calculation Urine Samples:** The result in "pg/mL" as read from the calibration curve must be multiplied by 100 to obtain the aldosterone concentration, in picograms per milliliter, of the original, unextracted urine sample. Divide this figure by 1,000, then multiply by the total volume in liters, to report the 24-hour aldosterone output in micrograms per day.

(A correction factor of 100 is used because the urine samples are twice diluted 1-in-10: first by extracting 0.25 mL urine into 2.5 mL ethyl acetate, then by reconstituting the residue of 0.1 mL in 0.5 mL of Incubation buffer (Saline Solution) and using 50 µL of it. The addition of hydrochloric acid during the hydrolysis step has no effect on the dilution.)
### Packaging Information Sheet

**Spiegazione dei simboli**
**Explanation of symbols**
**Explication des symboles**
**Significado de los símbolos**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>DE</th>
<th>ES</th>
<th>FR</th>
<th>GB</th>
<th>IT</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Symbol]</td>
<td>In vitro Diagnostikum</td>
<td>Producto sanitario para diagnóstico in vitro</td>
<td>Dispositif medical de diagnostic in vitro</td>
<td>In vitro Diagnostic Medical Device</td>
<td>Dispositivo medico-diagnostico in vitro</td>
<td>Dispositivos medicos de diagnostico in vitro</td>
</tr>
</tbody>
</table>

**Verwendete Symbole**
**Explicaçao dos simbolos**

<table>
<thead>
<tr>
<th>DE</th>
<th>ES</th>
<th>FR</th>
<th>GB</th>
<th>IT</th>
<th>PT</th>
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</thead>
<tbody>
<tr>
<td>Bestellnummer</td>
<td>Número de catálogo</td>
<td>Références du catalogue</td>
<td>Catalogue number</td>
<td>Numero di Catalogo</td>
<td>Número do catálogo</td>
</tr>
</tbody>
</table>

**Verwendbar bis**
**Establa hasta (usar antes de último día del mes)**
**Utiliser avant (dernier jour du mois indiqué)**
**Use by (last day of the month)**
**Utilizzare prima del (ultimo giorno del mese)**
**Utilizar (antes ultimo dia do mês)**

**Biogefährdung**
**Riesco biológico**
**Risque biologique**
**Biological risk**
**Rischio biologico**
**Risco biológico**

**Gebrauchsanweisung beachten**
**Consultar las instrucciones**
**Consulter le mode d’emploi**
**Consult instructions for use**
**Consultare le istruzioni per l’uso**
**Consultar instruções para uso**

**Ausreichend für “n” Tests**
**Contenido suficiente para “n” tests**
**Contenu suffisant pour “n” tests**
**Contains sufficient for “n” tests**
**Contenido suficiente para “n” testes**

**Temperaturbereich**
**Limitaciôn de temperatura**
**Limites de température de conservation**
**Temperature limitation**
**Limiti di temperatura**
**Temperaturas límites de conservação**
SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

**ERRORI CAUSE POSSIBILI/ SUGGERIMENTI**

Nessuna reazione colorimetrica del saggio
- mancata dispensazione del coniugato  
- contaminazione del coniugato e/o del Substrato  
- errori nell’esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

**Reazione troppo blanda (OD troppo basse)**
- coniugato non idoneo (es. non proveniente dal kit originale)  
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

**Reazione troppo intensa (OD troppo alte)**
- coniugato non idoneo (es. non proveniente dal kit originale)  
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta  
- qualità scadente dell’acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)  
- lavaggi insufficienti (coniugato non completamente rimosso)

**Valori inspiegabilmente fuori scala**
- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)  
CV% intrasaggio elevato  
- reagenti e/o strip non portate a temperature ambiente prima dell’uso  
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)  
CV% intersaggio elevato  
- condizioni di incubazione non costanti (tempo o temperatura)  
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)  
- variabilità intrinseca degli operatori

**ERROR POSSIBLE CAUSES / SUGGESTIONS**

No colorimetric reaction
- no conjugate pipetted reaction after addition  
- contamination of conjugates and/or of substrate  
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

**Too low reaction (too low ODs)**
- incorrect conjugate (e.g. not from original kit)  
- incubation time too short, incubation temperature too low

**Too high reaction (too high ODs)**
- incorrect conjugate (e.g. not from original kit)  
- incubation time too long, incubation temperature too high  
- water quality for wash buffer insufficient (low grade of deionization)  
- insufficient washing (conjugates not properly removed)

**Unexplainable outliers**
- contamination of pipettes, tips or containers  
insufficient washing (conjugates not properly removed) too high within-run  
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use  
- plate washer is not washing correctly (suggestion: clean washer head)  
too high between-run - incubation conditions not constant (time, CV % temperature)  
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)  
- person-related variation