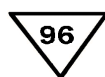


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**Instructions for use**  
**Aldosterone ELISA**

**REF**

**MS E-5200**



**IVD**

**CE**

## **Aldosterone ELISA**

### **1. Intended use and principle of the test**

For the direct quantitative determination of Aldosterone in human serum, plasma and urine by an enzyme immunoassay. For *in vitro* diagnostic use only.

The principle of the following enzyme immunoassay test follows 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 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 patient samples and controls can be directly read.

### **2. Procedural cautions and warnings**

This kit is intended for *in vitro* use only.

Practice the following good laboratory practices when handling kit reagents:

- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes; use safety glasses; in case of contact, flush 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.
- Avoid microbial contamination of reagents.
- A Standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (included in kit) should be included in every run and fall within established confidence limits.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- 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.
- 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.
- 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.
- 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.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- 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.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

### **3. Limitations**

- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

#### 4. **Safety cautions and warnings**

POTENTIAL BIOHAZARDOUS MATERIAL

All serum samples should be considered a potential biohazard and handled with the appropriate precautions.






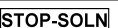







##### **Chemical hazards**

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.

#### 5. **Storage and stability**

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date shown on the kit labels.

#### 6a. **Reagents provided**

<b>MS E-5231</b>	 96	<b>Coated microtiter plate</b>	1	Rabbit Anti-Aldosterone Antibody Coated Microwell Plate - Break Apart Wells (12x8) - Ready To Use.
<b>MS E-5240</b>	 50x	<b>Conjugate Concentrate</b>	1 x 300 µl	Aldosterone-Horse Radish Peroxidase (HRP) Conjugate Concentrate. Has to be diluted 1 + 50 with assay buffer prior to use
<b>MS E-5213</b>		<b>Assay Buffer</b>	15 ml	One bottle containing a protein-based buffer with a non-mercury preservative - Ready To Use
<b>AA E-0030</b>	 10x	<b>Wash Buffer Concentrate</b>	50 ml	One bottle containing a buffer with a non-ionic detergent and a non-mercury preservative. Has to be diluted 1+9 with distilled water prior to use.
<b>AA E-0055</b>		<b>Substrate</b>	16 ml	One bottle containing tetramethylbenzidine and hydrogen peroxide - Ready To Use
<b>AA E-0080</b>		<b>Stopping Solution</b>	6 ml	One vial containing 1M sulfuric acid - Ready To Use
<b>MS E-5201</b>	 A	<b>Standard A</b>	1 x 2 ml	0-Standard, Ready To Use
<b>MS E-5202</b>	 B	<b>Standard B*</b>	1 x 0,6 ml	Ready To Use
<b>MS E-5203</b>	 C	<b>Standard C*</b>	1 x 0,6 ml	Ready To Use
<b>MS E-5204</b>	 D	<b>Standard D*</b>	1 x 0,6 ml	Ready To Use
<b>MS E-5205</b>	 E	<b>Standard E*</b>	1 x 0,6 ml	Ready To Use
<b>MS E-5206</b>	 F	<b>Standard F*</b>	1 x 0,6 ml	Ready To Use
<b>MS E-5251</b>		<b>Control</b>	1 x 0,6 ml	Ready To Use

\*) Listed above are approximate concentrations, please refer to vial labels for exact concentrations. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

#### 6b. **Additional materials and equipment required but not provided in the kit**

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.
6. Urine Diluent - Required if urine samples are to be analysed. Used for dilution of urine specimens before assaying. Has to be ordered separately (MS E-5241; 20 mL)

## 7. Sample 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.

## 8. Test procedure

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

### 8.1 **Sample preparation**

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 500 µl of urine diluent, add 10 µl of urine sample.

### 8.2 **Preparation of reagents**

#### **Conjugate**

Dilute the conjugate concentrate 1 + 50 in assay buffer before use (eg. 40 µl of concentrate 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.

#### **Wasch buffer**

Dilute the concentrate 1 + 9 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.

### 8.3 **Aldosterone ELISA**

1. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator
2. Pipette **50 µl** of each **calibrator, control and specimen sample** (serum or diluted urine) into correspondingly labelled wells in duplicate.
3. Pipette **100 µl** of the **conjugate working solution** into each well (We recommend using a multichannel pipette).
4. Incubate on a **plate shaker** (approximately 200 rpm) for **1 hour** at **room temperature**.
5. **Wash** the wells **3 times** with **300 µl** of 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).
6. Pipette **150 µl** of **TMB substrate** into each well.
7. Incubate on a **plate shaker** for **15-20 minutes** at **room temperature** (or until calibrator A attains dark blue colour for desired OD)
8. Pipette **50 µl** of **stopping solution** into each well.
9. **Read** the plate on a microwell plate reader **450nm** within 20 minutes after addition of the stopping solution.

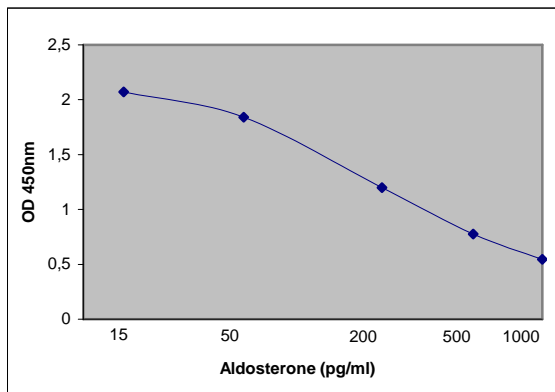
**9. Calculation of results**

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 samples directly off the calibrator 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  $1 \times 10^6$  to obtain values in  $\mu\text{g}/24$  hour.
6. If a serum or plasma sample 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 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 (example, do not use for your own calculation):

Standard	OD 1	OD 2	Mean OD	Concentration (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

Typical standard curve (example, do not use for your own calculation):



**10. Assay characteristics**

<b>Expected Reference Values</b>		<b>Aldosterone</b>
	Urine (normal salt intake)	5-19 $\mu\text{g}/24$ hours
	Serum (standing; normal salt intake) Serum (recumbent, normal salt intake)	40-310 pg/ml 10-160 pg/ml
<b>Analytical Sensitivity (Limit of Detection)</b>	<b>Aldosterone</b>	
	10 pg/ml	

	Substance	Cross reactivity (%)
		Aldosterone
<b>Analytical Specificity (cross-reactivity)</b>	11-Deoxycorticosterone	1.1
	Androsterone	< 0.001%
	Cortisone	< 0.001%
	11-Deoxcortisol	< 0.001%
	21-Deoxycortisol	< 0.001%
	Dihydrotestosteron	< 0.001%
	Estradiol	< 0.001%
	Estron	< 0.001%
	Testosterone	< 0.001%

**Precision** (values in pg/ml)

**Intra-Assay-Precision:**

Sample	mean	SD	CV%
1	18.79	1.96	10.4
2	128.67	5.26	4.1
3	507.22	27.44	5.4

**Inter-Assay-Precision:**

Sample	mean	SD	CV%
1	18.36	1.72	9.4
2	128.52	12.50	9.7
3	505.77	48.55	9.6












**Linearity** (values in pg/ml)

Sample	measured	expected	Recovery%
1	395.1	-	-
1:2	198.6	197.6	100.5
1:4	80.7	98.8	81.7
1:8	44.2	49.5	89.5
2	414.2	-	-
1:2	206.7	207.1	99.8
1:4	103.9	103.6	100.3
1:8	56.7	51.8	109.5

**Recovery** (values in pg/ml)

Sample	measured	expected	Recovery%
1 Unspiked	45.30	-	-
+51.0	119.1	96.3	123.7
+101.90	143.8	147.2	97.6
+203.80	227.5	249.1	91.3
2 Unspiked	130.0	-	-
+51.0	209.4	181.0	115.7
+101.90	243.1	231.9	104.8
+203.80	307.5	333.8	92.1
3 Unspiked	208.4	-	-
+51.0	289.3	259.4	111.5
+101.90	341.6	310.3	110.1
+203.80	460.1	412.2	111.6

**Symbols:**

	Enthält Testmaterial für <n> Teste		Hersteller		Lagertemperatur
	Katalog-Nummer		Chargennummer		Verwendbar bis
	In vitro Diagnostikum		Inhalt		Vor Gebrauch Packungsbeilage lesen
	Nur für Forschungszwecke		Achtung		