DRG® Aldosterone ELISA (EIA-4410)

RUO in the USA

REVISED 17 JAN. 2011 RM (VERS. 4.1)

This kit is intended for Research Use Only.
Not for use in diagnostic procedures.
Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

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

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2 PRINCIPLE

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 samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites 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 addiction 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 samples and controls can be directly read.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **Standards** (6 vials, 1 mL each)
   STD0 - STD5
2. **Conjugate** 50X (1 vial, 1 mL)
   Aldosterone-HRP conjugate
3. **Coated Microplate** (1 microplate breakable)
   coated with anti-Aldosterone
4. **Incubation Buffer** (1 vial, 30 mL)
   Phosphate buffer 50 mM pH 7.5; BSA 1 g/L; stabilizer
5. **50X Conc. Wash Solution** (1 vial, 20 mL)
   NaCl 45 g/L; Tween-20 55 g/L
6. **TMB Substrate** (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L (avoid any skin contact)
7. **Stop Solution** (1 vial, 15 mL)
   Sulfuric acid, 0.15 mol/L (avoid any skin contact)

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note
Store all reagents between 2 °C – 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

- The reagents contain Proclin 300^R as preservative.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lots of reagents.
- Do not use heavily haemolized samples.
- This method allows the determination of Aldosterone from 20 to 2000 pg/mL
- The treatment with natural or synthetic steroids can affect blood levels of aldosterone.

5 PROCEDURE

5.1 Preparation of the Standards (S0, S1, S2, S3, S4, S5)

The standards have approx. the following concentrations:

	S0	S1	S2	S3	S4	S5
pg/mL	0	20	80	300	800	2000

Once opened, the standards are stable for six months at +4 °C.

5.2 Preparation of Wash Buffer

Dilute the content 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 °C – 8 °C.

5.3 Preparation of Diluted Conjugate

Prepare immediately before use.

Dilute the Conjugate 1:50 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 (Standard 0).
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 (S0-S5), and for each sample, one for Blank.
Pipette:

Reagent	Standard	Samples	Blank
Standard S0-S4	50 µL		
Samples		50 µL	
Diluted Conjugate	100 µL	100 µL	
Incubate at +37 °C for 1 hour. Remove the contents from each well; wash the wells 3 times with 300 µL of diluted wash solution.			
TMB substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22 °C – 28 °C) for 20 minutes in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank			

6 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 micro pipette. 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 re-suspend the extract by vortexing.
10. Transfer **50 µL of Re-suspend to the well of coated microplate**

Proceed with the assay procedure, as described in the IFU (using the re-suspended 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.

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