Aldosterone

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55111-96

www.zellx.de

Sample Types Validated for:

Serum, EDTA and Heparin Plasma, Urine, Saliva, Fecal Extracts, and Tissue Culture Media

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

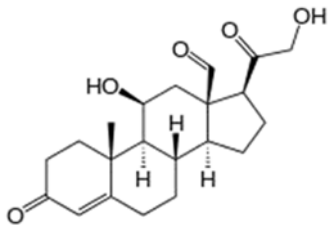
Please read this insert completely prior to using the product.

Introduction

Background

Aldosterone ($C_{21}H_{28}O_3$) is a mineralocorticoid hormone produced by aldosterone synthase enzyme (CYP11B2) in the zona glomerulosa of the adrenal cortex. It controls the sodium-potassium balance via the unidirectional salt reabsorption in a variety of tissues and glands including kidney and colon. Aldosterone, together with Renin and Angiotensin, create a hormone system called Renin-Angiotensin-Aldosterone system (RAAS). RAAS regulates the blood stream in kidney in response to rehydration due to a reduced blood pressure, or a significant drop in blood volume after a hemorrhage or serious injury. It also indirectly affects the blood's pH and electrolyte levels. Being dependent on age and body position, peripheral Aldosterone levels are typically less than 300 pg/mL in a normal upright adult.

Aldosterone measurement is useful in the diagnosis of primary aldosteronism (i.e., adrenal adenoma or carcinoma and adrenal cortical hyperplasia) and secondary aldosteronism (renovascular disease, salt depletion, potassium loading, cardiac failure with ascites, pregnancy, Bartter syndrome). The Renin-Angiotensin system is the primary regulator of the synthesis and secretion of Aldosterone. Increased concentrations of potassium in the plasma may directly stimulate adrenal production of the hormone.



Assay principle

The ZellX® Aldosterone Immunoassay kit has been designed to quantitatively measure the amount of Aldosterone present in extracted serum, plasma, urine, saliva, extracted dried fecal samples, as well as tissue culture media samples. An Aldosterone stock solution is provided to generate a standard curve for the assay, and all samples should be read off the standard curve.

The kit includes a 96-well plate pre-coated with a secondary anti-sheep antibody. The function of this antibody is to capture the sheep anti-Aldosterone antibody bound to Aldosterone conjugate (peroxidase-labeled), and hold this complex to the plate during the subsequent detection steps. The Aldosterone-conjugate (labeled) and the sample Aldosterone (unlabeled) compete for binding to the sheep antibody. After overnight incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of Aldosterone in the sample, the stronger the signal is due to more labeled Aldosterone bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Aldosterone Standard (40 ng/mL)	125 µL
Aldosterone Antibody	2.6 mL
Aldosterone Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For Dried Fecal, Serum and Plasma Sample:

ACS Grade Ethanol or Ethyl Acetate

Glass test tubes

Speedvac

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

Since Aldosterone is identical across all species, it is expected that this kit can measure Aldosterone in human and other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

All samples and standards must be used within 2 hours of preparation or must be stored at ≤ -20 for later analysis.

I. **Serum, Plasma:**

- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Separate the serum or plasma from the red blood cells, and transfer into fresh tubes.
- Add 250 µL of serum or plasma to a glass test tube and add 250 µL of Ethyl Acetate.
- Vortex gently and allow layers to separate. Gently draw off the top organic layer and place it in a clean tube.
- Repeat the extraction with Ethyl Acetate 2 more times, pooling the Ethyl Acetate supernatants.
- Speedvac the Ethyl Acetate supernatant to dryness.
- Reconstitute with 10 µL of Ethanol and dilute with 240 µL of supplied Assay Buffer.
- This dilution can be diluted further with Assay Buffer.

II. **Urine:**

- Urine should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting the assay.
- **Normalize the sample value based on Creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in a random urine specimen.**

III. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
- Add 1 mL of Ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

IV. **Saliva:**

- Freeze the collected saliva at -20°C.
- Upon thawing, centrifuge it at 2500 g for 20 minutes and collect the clear supernatant. Analyze collected supernatant immediately or aliquot and freeze at -20°C.
- Saliva should be diluted ≥ 1:2 by taking one part of sample and adding 1 or more parts of Assay Buffer prior to conducting the assay.

V. **Tissue Culture Media:**

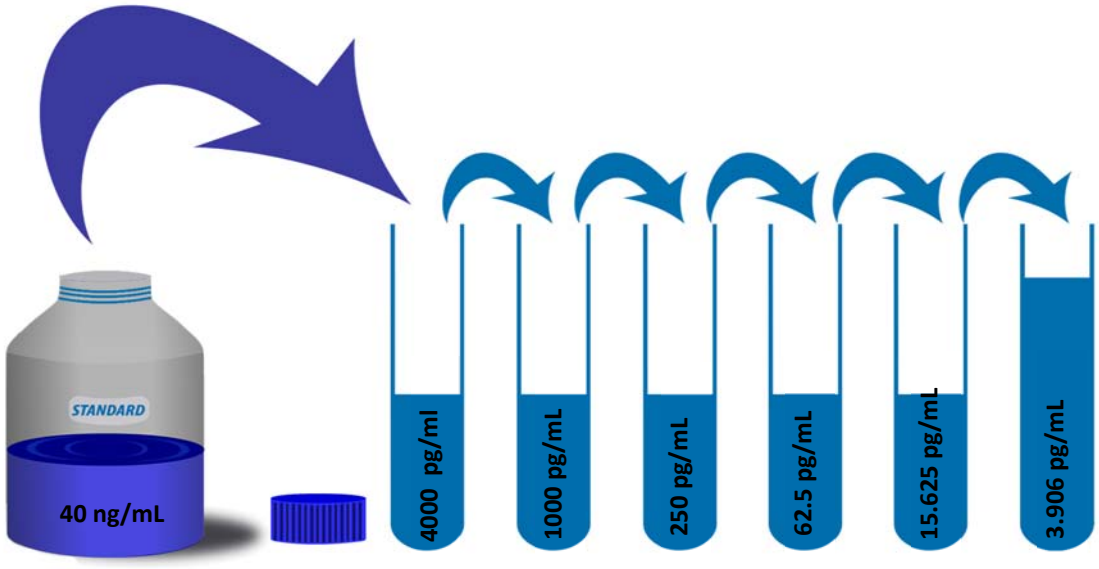
- For measuring Aldosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All samples must be used within 2 hours of preparation; otherwise, aliquots of the sample should be kept at ≤ -20˚C for later use.

Standard preparation

- Prepare a 1:10 dilution of Aldosterone Standard with Assay Buffer (mix 40 µL of Standard with 360 µL of Assay Buffer), and label as the Standard No.6 (4000 pg/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	4000 pg/mL	40 µL Aldosterone Standard + 360 µL Assay Buffer
Standard No.5	1000 pg/mL	100 µL Standard No.6 + 300 µL Assay Buffer
Standard No.4	250 pg/mL	100 µL Standard No.5 + 300 µL Assay Buffer
Standard No.3	62.5 pg/mL	100 µL Standard No.4 + 300 µL Assay Buffer
Standard No.2	15.625 pg/mL	100 µL Standard No.3 + 300 µL Assay Buffer
Standard No.1	3.906 pg/mL	100 µL Standard No.2 + 300 µL Assay Buffer
Standard No.0	0 pg/mL	300 µL Assay Buffer



All standards must be used within 2 hours of preparation

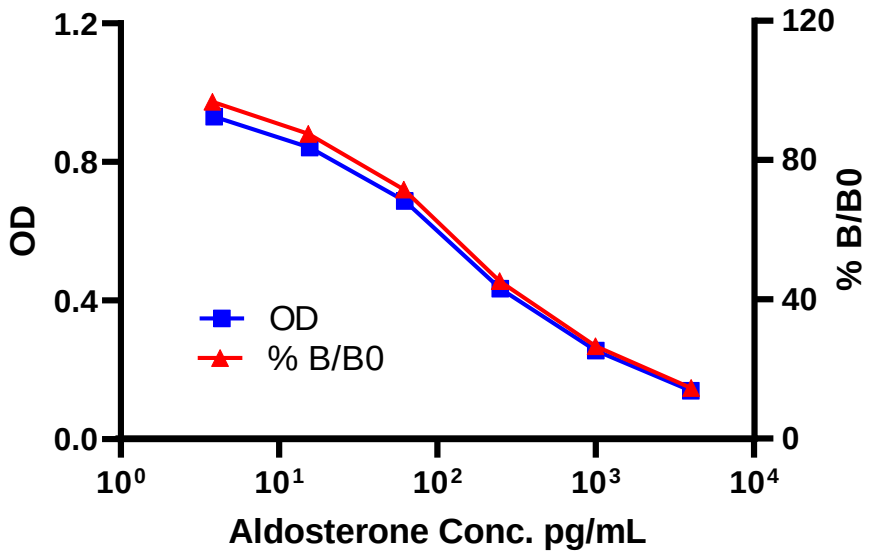
Assay Procedure

1. Pipette 100 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 100 µL of Assay Buffer into duplicate wells of the Zero standard (maximum binding wells).
3. Pipette 125 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Aldosterone Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Aldosterone Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 15 minutes at room temperature, followed by overnight incubation at 4°C without shaking.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate (equilibrated to room temperature) to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes without shaking.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 pg/mL of Aldosterone is equivalent to 277.4 pM



A typical standard curve of ZellX® Aldosterone ELISA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Aldosterone ELISA assay was determined as 14.76 pg/mL.

Sensitivity

The sensitivity of the ZellX® Aldosterone ELISA assay was determined as 4.97 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 17 different assays over multiple days.

Item	*%CV*
Intra assay	5.9, 8.8, 6.0
Inter assay	12.2, 25.8, 20.5

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	*Cross Reactivity (%)*
Aldosterone	100%
Corticosterone	0.047%
Desoxycorticosterone	0.019%
Progesterone	<0.016%
Tetrahydrocorticosterone	<0.016%
Cortisol	<0.016%
1-dehydroCortisol	<0.016%
Estradiol	<0.016%

Protocol summary

Add 100 µL samples/standard into duplicate well

↓

Add 100 µL Assay Buffer into duplicate well as zero

↓

Add 125 µL Assay Buffer into NSB

↓

Add 25 µL Aldosterone Conjugate into each well

↓

Add 25 µL Aldosterone Antibody into each well except NSB

↓

Tap the side of the plate, mix well, Incubate 15 min at RT without shaking, followed by overnight incubation at 4° C

↓

Aspirate the plate & wash all wells 4 times with 300 µL wash buffer

↓

Add 100 µL of the TMB Substrate to each well

↓

Incubate 30 min at RT

↓

Add 50 µL Stop Solution to each well

↓

Read the absorbance at 450 nm

References

1. Williams, J.S., & Williams, G.H. (2003). 50th anniversary of aldosterone. *The Journal of Clinical Endocrinology& Metabolism, 88*(6), 2364-2372.

2. Rogerson, F.M., & Fuller P.J. (2000). Mineralocorticoid action. *Steroids, 65*(2), 61-73.

3. Agarwal, M.K., & Mirshahi, M. (1999). General overview of mineralocorticoid hormone action. *Pharmacology & Therapeutics, 84*(3), 273-326.

4. Lumbers, E. R. (1999). Angiotensin and aldosterone. *Regulatory Peptides, 80*(3), 91-100.

5. Cartledge, S., & Lawson, N. (2000). Aldosterone and renin measurements. *Annals of Clinical Biochemistry, 37*(3), 262-278.

6. Loeuille, G.A., et al. (1981). Blood and urinary aldosterone levels in normal neonates, infants and children. *Pediatrie, 36*(5), 335-344.