Version 6c Last updated 10 October 2023

ab136933
Aldosterone ELISA Kit

For quantitative detection of Aldosterone in plasma, serum and urine.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's Aldosterone *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Aldosterone in plasma, serum and urine.

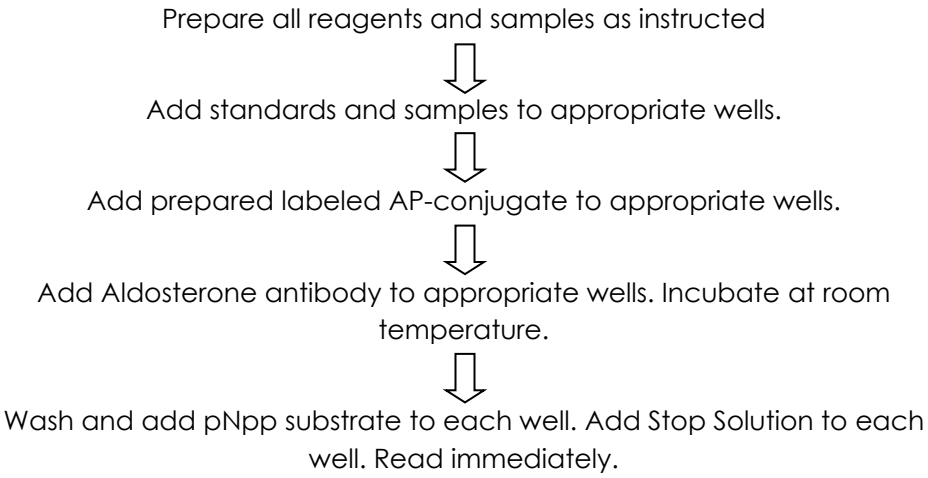
A donkey anti-sheep IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated- Aldosterone antigen and a polyclonal sheep antibody specific to Aldosterone. After incubation, the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of Aldosterone captured in the plate.

Aldosterone is a steroid hormone synthesized from cholesterol in the adrenal cortex. Aldosterone is metabolized in the kidney and liver, and functions as the key mineralocorticoid in the control of sodium and potassium balance. Synthesis and release of aldosterone by the adrenal gland is primarily regulated by the renin-angiotensin-alsdosterone system (RAAS), the main regulatory system involved in blood pressure regulation, renal hemodynamics, and sodium-volume homeostasis. Measurement of serum aldosterone in conjunction with plasma renin is used clinically to differentiate between primary and secondary aldosteronism. Primary aldosteronism (hyperaldoseronism) is characterized by a very low renin: aldosteone ratio leading to the retention of sodium and increased blood pressure, and is typically the result of renal gland hyperplasia or tumors. In secondary aldosteronism, hyperproduction of aldosterone results from external conditions such as heart failure and renal artery disease that reduce renal blood flow and stimulate the RAAS mechanism.

The RAAS directly affects vascular and cardiac remodeling through proliferative and inflammatory signaling, as aldosterone and salt have been shown to increase the expression of intracellular cyclooxygenase-2, osteopontin, and MCP-1 in rats. Aldosterone acts by binding to the mineralocorticoid receptor (MR) triggering the transcription of hormone responsive genes, and clinical studies have shown that patients with congestive heart failure or after myocardial infarction benefited from MR antagonist treatment. As pharmacological modulation of nuclear hormone receptors is a common strategy for the treatment of cardiovascular disease, determining the effect of such treatments on

the RAAS is of increasing value in evaluating the safety and efficacy of new targeted therapeutics.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Donkey anti-sheep IgG Microplate (12 x 8 wells)	96 wells	+4°C
Aldosterone Alkaline Phosphatase Conjugate	5 mL	-20°C
Aldosterone Antibody	5 mL	-20°C
Aldosterone Standard	0.25 mL	-20°C
Assay Buffer	27 mL	+4°C
20X Wash Buffer Concentrate	27 mL	+4°C
pNpp Substrate	20 mL	+4°C
Stop Solution	5 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- Eppendorf tubes.
- Microplate Shaker.
- Absorbent paper for blotting.
- 1,000 mg C18 Solid Phase Extraction Columns (only required for extraction of samples containing low levels of Aldosterone).
- 0.2N hydrochloric acid (only required for extraction of samples containing low levels of Aldosterone).
- Deionized water.
- 100% Methanol.
- Diethyl ether.
- Speedvac.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
- The standard should be handled with care due to the known and unknown effects of the antigen.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Conjugate 1:5 Dilution for Total Activity Measurement

Prepare the Conjugate 1:5 Dilution by diluting 20 µL of the supplied Conjugate with 80 µL of the assay buffer. The dilution should be made after the overnight incubation. This 1:5 dilution is intended for use in the Total Activity wells only.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Reconstitution of the Aldosterone standard should be prepared no more than 1 hour prior to the experiment.
- Diluted standards should be used within 60 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Allow the reconstituted 10,000 pg/mL Aldosterone Stock Standard solution to equilibrate to room temperature.

10.2 Label 7 tubes #1 – #7.

10.3 Add 975 µL Assay Buffer into tube #1.

10.4 Add 500 µL Assay Buffer into tubes #2 to #7.

10.5 Prepare a 250 pg/mL **Standard 1** by adding 25 µL of the 10,000 pg/mL Stock Standard to tube #1. Mix thoroughly and gently.

10.6 Prepare **Standard 2** by transferring 500 µL from Standard 1 to tube #2. Mix thoroughly and gently.

10.7 Prepare **Standard 3** by transferring 500 µL from Standard 2 to tube #3. Mix thoroughly and gently.

10.8 Using the table below as a guide, repeat for tubes 4 through 7.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	25 µL Standard	975	10,000	250
2	500 µL Standard #1	500	250	125
3	500 µL Standard #2	500	125	62.5
4	500 µL Standard #3	500	62.5	31.3
5	500 µL Standard #4	500	31.3	15.6
6	500 µL Standard #5	500	15.6	7.8
7	500 µL Standard #6	500	7.8	3.9

11. Sample Preparation

The Aldosterone kit is compatible with Aldosterone samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples.

Some samples normally have very low levels of Aldosterone present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

11.1 Protocol for Urine samples:

11.1.1 Centrifuge Urine at ~20,000 x g for 5 minutes at 4 °C.

11.1.2 Transfer 1 part supernatant to a tube and add 2 parts of 0.2 N HCl (e.g. 500 µL urine + 1 mL 0.2 N HCl).

11.1.3 Leave overnight at room temperature, capped and protected from light.

11.2 Protocol for Serum/ Plasma samples (optional):

11.2.1 Condition 1000 mg C 18 solid phase system columns on a vacuum manifold by passing 5-10 mL of 100% methanol through the columns, followed by 5-10 mL of dH_2O.

11.2.2 Apply Serum and Plasma samples.

11.2.3 Wash columns with 5-10 mL dH_2O. Allow water to drain completely from columns until dry.

11.2.4 Elute samples with 2 mL of diethyl ether.

11.2.5 Dry samples down in a speed vac for 2-3 hours

11.2.6 Rehydrate samples at room temperature in the assay buffer. A minimum of 250 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

Recommended plate layout

	1	2	3	4
A	B_s	Std 1	Std 5	Sample 2
B	B_s	Std 1	Std 5	Sample 2
C	TA	Std 2	Std 6	etc
D	TA	Std 2	Std 6	etc
E	NSB	Std 3	Std 7	
F	NSB	Std 3	Std 7	
G	B_0	Std 4	Sample 1	
H	B_0	Std 4	Sample 1	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

Key:

B_s = Blank; contains substrate only.

TA = Total Activity; contains conjugate (5 µL) and substrate.

NSB = Non-specific binding; contains assay buffer 1, conjugate and substrate.

B_0 = 0 pg/mL standard; contains assay buffer 1, conjugate, antibody and substrate.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Refer to the recommended plate layout in Section 12 before proceeding with the assay.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 150 µL of the Assay Buffer into the NSB (non specific binding) wells.

13.3 Add 100 µL of the Assay Buffer into the BO (0 pg/mL standard) wells.

13.4 Add 100 µL of prepared standards and 100 µL diluted samples to appropriate wells.

13.5 Add 50 µL of Aldosterone Alkaline Phosphatase Conjugate (blue) to NSB, B0, standards, and samples i.e. not the TA (Total Activity) and Bs (blank) wells.

13.6 Add 50 µL of Aldosterone Antibody (yellow) to B0, standard and sample wells, i.e. not Bs, TA and NSB wells.

13.7 Note: Every well used should be green except the NSB wells which should be blue. The Bs and TA wells are empty at this point and have no color.

13.8 Seal the plate. Incubate the plate at 4°C overnight without shaking.

13.9 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.10 Add 5 µL 1:5 diluted Aldosterone Alkaline Phosphatase Conjugate (blue) to the TA wells.

13.11 Add 200 µL of the Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.

13.12 Add 50 µL Stop Solution into each well. The plate should be read immediately.

13.13 After blanking the plate reader against the Bs (blank) wells, read optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked

against the Bs wells, manually subtract the mean optical density of the blank wells from all readings.

14. Calculations

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average Bound OD - Average NSB OD

14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (B_0), using the following formula:

Percent Bound = (Average Net OD/Average Net B_0 OD) x 100

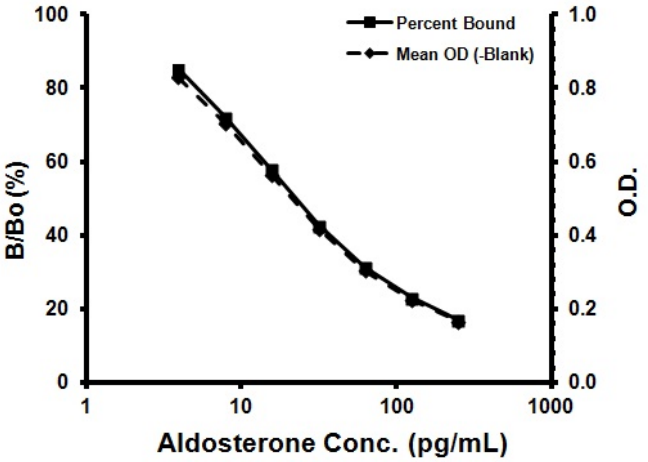
14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of aldosterone for the standards. The concentration of aldosterone in the unknowns can be determined by interpolation.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Sample	Mean OD (-Blank)	% Bound	Aldosterone (pg/mL)
TA	0.524		
NSB	0.000	0	
Standard 1	0.165	16.9	250
Standard 2	0.225	23.1	125
Standard 3	0.304	31.2	62.5
Standard 4	0.418	42.7	31.3
Standard 5	0.566	58.0	15.6
Standard 6	0.704	72.1	7.8
Standard 7	0.829	85.1	3.9
B_0	0.976	100	0
Unknown 1	0.315	32.3	58.4
Unknown 2	0.669	68.5	9.4

Figure 1. Example of Aldosterone standard curve.

16. Typical Sample Values

SENSITIVITY –

The sensitivity, minimum detectable dose of Aldosterone using this Abcam ELISA kit was found to be 4.7 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 7. The detection limit was determined as the concentration of Aldosterone measured at two standard deviations from the zero along the standard curve.

LINEARITY OF DILUTION –

Human samples containing serotonin were serially diluted 1:2 in the assay buffer and measured in the assay. Results are shown in the table below. Human and rat samples containing Aldosterone were serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

	Average % Of Expected				
Dilution	Human Plasma	Rat Plasma	Rat Serum	Human Serum	Human Urine
Neat	-	-	108	-	-
1:2	89	105	108	-	-
1:4	95	102	109	-	-
1:8	92	84	121	93	-
1:16	100	86	122	96	107
1:32	-	98	90	98	103
1:64	-	109	103	100	100

SAMPLE RECOVERY –

After diluting each sample matrix, aldosterone standard was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean of percent recovery at the three concentrations are indicated below for each matrix.

Sample	Dilution	Spike Concentration (pg/mL)	Mean % Recovery
Human Serum	1:16	120.0	75.7
		30.0	76.1
		7.5	57.6
Human Plasma	1:8	120.0	88.1
		30.0	112.5
		7.5	124.2
Human Urine	1:32	120.0	124.8
		30.0	94.2
		7.5	77.1

PRECISION –

Intra-assay

	Aldosterone (pg/mL)	%CV
Low	13.9	6.6
Medium	27.7	4.4
High	84.8	4.5

Inter-assay

	Aldosterone (pg/mL)	%CV
Low	10.8	16.3
Medium	24.4	18.0
High	72.6	10.8

17. Assay Specificity

CROSS REACTIVITY –

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of 100, 10, 1, and 0.1 times the high standard. These samples were then measured in the assay:

Compound	% cross reactivity
11-Deoxycorticosterone	0.30 %
Progesterone	0.20 %
Corticosterone	0.19 %
Cortisol	< 0.001 %
DHT	< 0.001 %
Estradiol	< 0.001 %
Testosterone	< 0.001 %

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)