Aldosterone ELISA Kit

08/17

(Catalog # E4342-100, 100 assays, Store at 4°C)

I. Introduction:

Aldosterone controls the sodium-potassium balance through the unidirectional salt reabsorption in a variety of tissues and glands. Synthesized from cholesterol in the zona glomerulosa of the adrenal cortex, secretion is regulated through the renin-angiotensin system4. Angiotensin II and potassium stimulate primary secretion by increasing the rate of production of the steroid. Peripheral aldosterone levels are dependant on age and body position and in a normal upright adult aldosterone levels are typically less than 300 pg/ml. Aldosterone is typically secreted as the 18-glucuronide and the terahydro-3-glucuronide5 and this excretion is generally 2-20 µg/24 hour urine collection. BioVision's Aldosterone ELISA kit is a competitive ELISA assay for the quantitative measurement of Aldosterone in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Aldosterone.
Detection Range: 4000 – 3.9 pg/ml
Sensitivity: < 4.97 pg/ml

III. Specificity:

Universal

IV. Sample Type:

Extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue

V. culture media samples Kit Contents:

Components	E4342-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4342-100-1
Standard	125 µl	E4342-100-2
Aldosterone Antibody	3 ml	E4342-100-3
Aldosterone Conjugate	3 ml	E4342-100-4
Assay Buffer Concentrate (5X)	28 ml	E4342-100-5
Wash Buffer Concentrate (20X)	30 ml	E4342-100-6
TMB Substrate	11 ml	E4342-100-7
Stop Solution	5 ml	E4342-100-8
Plate Sealer	1	E4342-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

The entire kit may be stored at 4°C for up to. Avoid freeze-thaw cycles.

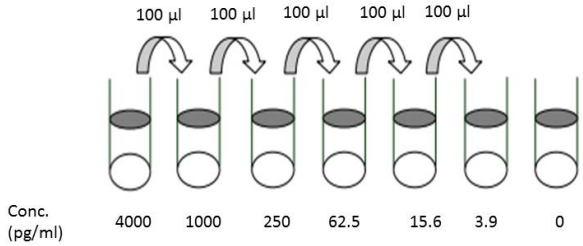
VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Assay Buffer:** Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.
2. **Wash Buffer**: Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.

3. **Standard Preparation:**

- Add 40 µl of the aldosterone stock solution to 360 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 4 vials of standards (tube #2-6) by adding 0.1 ml of the above stock solution in 0.3 ml of Assay Buffer. Perform 4-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 4,000, 1,000, 250, 62.5, 15.6, and 3.9 pg/ml.



FOR RESEARCH USE ONLY! Not to be used on humans.

4. Sample Preparation:

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Serum**: 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.
- **Urine**: Urine samples should be diluted ≥ 1:4 with the supplied Assay Buffer prior running in the assay.
- **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.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 100 µl of samples or standards into wells in the plate. Pipet 125 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the Aldosterone Conjugate to each well. Add 25 µl of the Aldosterone Antibody to each well, except the NSB wells.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 15 minutes. Store the sealed plate at 4°C overnight.
5. The next day, bring TMB substrate to room temperature 30 minutes prior to the assay.
6. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
7. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes.
8. Add 50 µl of the Stop Solution to each well.
9. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the non-specific binding well (NSB). The sample concentrations obtained, calculated from the $\%B/B_0$ curve, and should be multiplied by the dilution factor to obtain neat sample values.

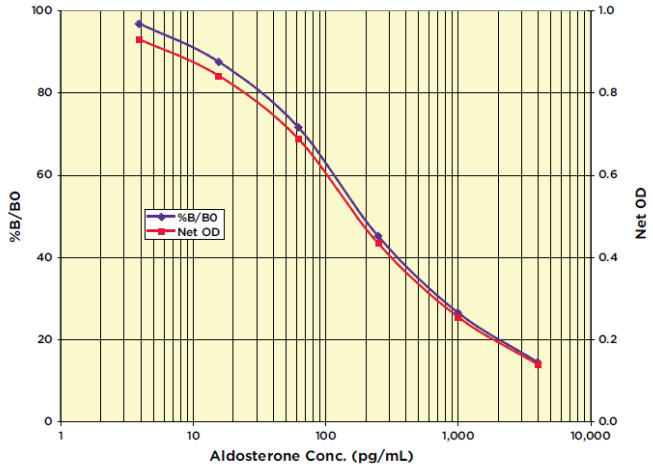


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. VALIDATION DATA:

Intra Assay:

Sample	Aldosterone Conc. (pg/mL)	%CV
1	1,018.7	6.0
2	156.2	5.9
3	40.6	8.8

Inter Assay Precision:

Sample	Aldosterone Conc. (pg/mL)	%CV
1	1,051.9	20.5
2	150.2	12.2
3	39.6	25.8

Cross Reactivity:

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%

XII. RELATED PRODUCTS:

- Human CellExp™ SERPIN A8, human recombinant (Cat. No. 7236)
- QuickDetect™ Corticosterone (Human) ELISA Kit (Cat. No. K4431)
- Progesterone (human) ELISA Kit (Cat. No. K7414-100)
- Cortisol (human/mouse/rat) ELISA Kit (Cat. No. K7430-100)
- Evacetrapib (Cat. No. 2558)
- BNP, human (Cat. No. 4875)

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