Aldosterone competitive ELISA

Catalog Number	Kit Size
TBS31001-01	1x 96-well Plate
TBS31001-05	5x 96-well Plate

INTRODUCTION

Aldosterone is a mineralocorticoid first isolated from beef adrenal glands. It controls the sodium-potassium balance through the unidirectional salt reabsorption in a variety of tissues and glands. Peripheral aldosterone levels are dependent on age and body position with an expected aldosterone level of less than 300 pg/mL being found in a typical upright adult.

Aldosterone measurement is useful in the investigation 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).

TribioScience's Aldosterone ELISA kit is a competitive ELISA assay for the quantitative measurement of aldosterone in extracted serum, extracted plasma, urine, extracted dried fecal samples, and tissue culture media samples.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	Catalog Number	
		TBS31001-01	TBS31001-05
Aldosterone Microplate	TBS31001A	1x 96-well microplate (12 strips of 8 wells) coated with a polyclonal anti-sheep IgG antibody.	5x 96-well microplate (12 strips of 8 wells) coated with a polyclonal anti-sheep IgG antibody.
Aldosterone Standard	TBS31001B	1x 125µL, 40,000 pg/mL in a special stabilizing solution.	5x 125µl, 40,000 pg/mL in a special stabilizing solution.
Detect A	TBS31001C	1x 3mL, An aldosterone-peroxidase conjugate in a special stabilizing solution.	5x 3mL, An aldosterone-peroxidase conjugate in a special stabilizing solution.
Detect B	TBS31001D	1x 3mL, Polyclonal antibody specific for Aldosterone.	5x 3mL, Polyclonal antibody specific for Aldosterone.
Assay Diluent	TBS31001E	1x 2mL, 5x concentrate Assay Diluent.	1x 10mL, 5x concentrate Assay Diluent.
Wash Buffer Concentrate	TBS3000W	1x 10mL, concentrated solution (10x).	1x 50mL, concentrated solution (10x).
TMB Substrate	TBS3000T	1x 12mL, ultra-sensitive TMB substrate.	5x 12mL, ultra-sensitive TMB substrate.
Stop Solution	TBS3000S	1x 6mL, 2N sulfuric acid.	1x 30mL, 2N sulfuric acid.

Store the unopened kit at 2-8 ℃. Do not use kit past expiration date.

OTHER MATERIALS NOT PROVIDED IN THE KIT

Distilled or deionized water.
Ethyl acetate or ethanol for serum, plasma or fecal extracts.
A speedvac for evaporation of ethanol or ethyl acetate
Repeater pipet with disposable tips capable of dispensing 25, 50, and 100 µL.
A microplate shaker.
Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 10mL of Wash Buffer Concentrate (10x) to 90mL of deionized water to prepare 100mL of Wash Buffer.

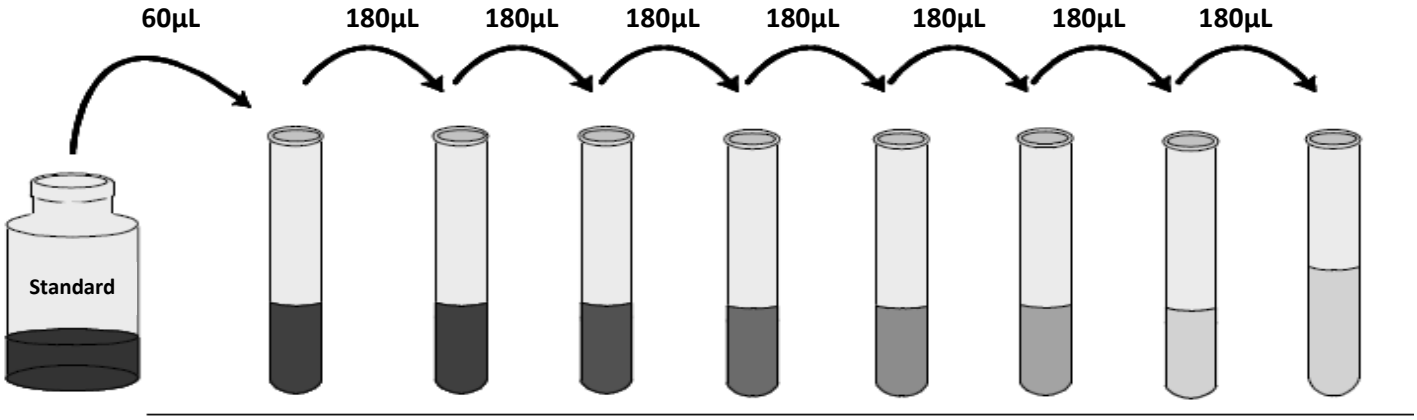
Assay Diluent: Dilute Assay Buffer Concentrate 1:5 by adding 8ml distilled water to 2mL of 5x Assay Diluent.

Standard preparation

1 Label test tubes as #1 through #8. Pipet 420µL of 1x Assay Buffer into tube #1 and 270µL into tubes #2 to #8. The aldosterone stock solution contains an organic solvent. *Note: The aldosterone stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery*.

2. Carefully add 60µL of the aldosterone stock solution to tube #1 and vortex completely.

3. Take 180µL of the aldosterone solution from tube #1, and add it into tube #2 and vortex completely. Then, repeat the serial dilutions from tubes #3 through #8. The concentration of aldosterone will be 5,000, 2000, 800, 320, 128, 51.2, 20.48 and 8.192 pg/mL. *Note: Use all Standards within 2 hours of preparation.*



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	420	270	270	270	270	270	270	270
Stock (40,000pg/mL)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Vol of Stock (µL)	60	180	180	180	180	180	180	180
Final Conc (pg/mL)	5,000	2,000	800	320	128	51.2	20.48	8.192

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate to allow the end user to accurately determine aldosterone concentrations.

For Serum and Plasma Samples:

Add 250µL of serum or plasma to a glass test tube and add 250µL 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 10µL of ethanol and dilute with 240µL of 1x Assay Buffer. This dilution can be diluted further with the Assay Buffer. Use all Samples within 2 Hours of preparation or stored at ≤ -20°C until assaying.

1. Pipet 100µL of samples or standards into wells in the plate.
2. Pipet 100µL of 1x Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
3. Pipet 125µL of 1x Assay Buffer into the non-specific binding (NSB) wells.
4. Add 25µL of the Detect A (Aldosterone Conjugate) to each well using a repeater pipet.

5. Add 25µL of the Detect B (Aldosterone Antibody) to each well, except the NSB wells, using a repeater pipet.
6. 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.
7. Store the sealed plate at 4°C overnight.
8. In the following day, remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. *Note: Addition of cold Substrate will cause depressed signal.*
9. Aspirate the plate and wash each well 3 times with 300µL wash buffer, 3 mins each time. Tap the plate dry on clean absorbent towels.
10. Add 100µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

(*NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells*).

CACULATION DATA

Average the duplicate readings for each standard, zero standard (B0), samples, NSB, and subtract the average optical density of NSB. Calculate the Maximum Binding Percent as the formula: % B/B0=100*(OD of B /OD of B0 (B: Sample or Standard. Create a standard curve by using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting % B/B0 for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed the value listed in the Table1.

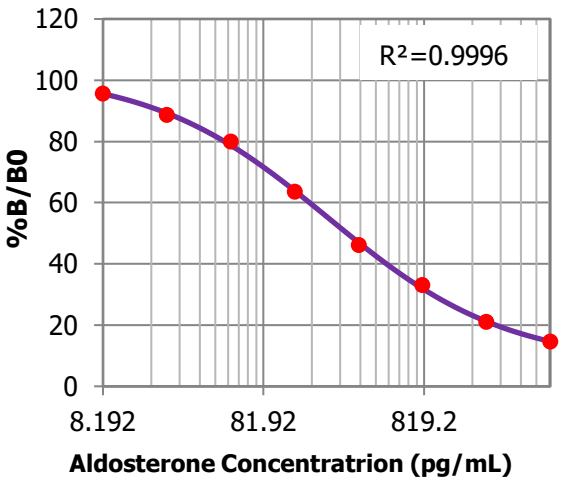


Table 1 TYPICAL DATA FOR OVERNIGHT PROTOCOL

Sample	Mean OD	Net OD	%B/B0	Aldosterone Con (pg/mL)
NSB	0.058	0.000		
Standard 1	0.194	0.136	14.5	5000
Standard 2	0.255	0.197	21.0	2000
Standard 3	0.367	0.309	33.0	800
Standard 4	0.491	0.432	46.1	320
Standard 5	0.653	0.595	63.5	128
Standard 6	0.807	0.749	79.9	51.2
Standard 7	0.888	0.830	88.6	20.48
Standard 8	0.954	0.896	95.6	8.192
B0	0.995	0.937	100	0

SENSITIVITY

Limit of Detection (LOD): The LOD for the assay is determine by the mean of OD from 6 wells run for each of the B0 minus two (2) standard deviations (SD). The LOD was 6.752 pg/mL in the assay.

SPECIFICITY

This assay recognizes natural and recombinant Aldosterone displayed as Table2.

RECOVERY

The recovery of Aldosterone spiked to levels throughout the range of the assay in various matrices listed as Table 3.

Table 2. Specificity of the Assay

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%

Table 3 Recovery in different samples

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	94	89-99%
Serum (n=4)	92	86-112%
EDTA plasma (n=4)	91	87-115%
Heparin plasma (n=4)	89	79-107%
Urine (n=4)	101	95-106%

Note: Always run your own standard curve for calculation of results. Do not use this data.
Conversion Factor: 100 pg/mL of aldosterone is equivalent to 277.4 pM.

VALIDATION DATA

Intra Assay Precision

Samples were assayed in duplicates in 16 runs by four operators to determine precision between assays in Table 4.

Inter Assay Precision

Samples were assayed in replicates of 20 to determine precision within assay in Table 5.

Table 4 Intra-assay precision

Parameters	Sample 1	Samples 2	Samples 3
Mean (pg/mL)	1018.7	156.2	40.6
%CV	6	5.9	8.8

CV = Coefficient of Variation

Table 5 Inter-assay precision

Parameters	Sample 1	Samples 2	Samples 3
Mean (pg/mL)	1051.9	150.2	39.6
%CV	20.5	12.2	25.8

CV = Coefficient of Variation

For research use only. Not for use in diagnostic procedures.

REFERENCES

1. Rogerson FM, and Fuller PJ. “Mineralocorticoid action” Steroids, 2000, 65: 61-73.
2. Cartledge S, and Lawson N. “Aldosterone and renin measurements.” Ann. Clin. Biochem., 2000, 37 (Pt. 3): 262-278.
3. Loeuille GA, Racadot A, Vasseur P, Vandewalle B. “Blood and urinary aldosterone levels in normal neonates, infants and children.” Pediatrie, 1981, 36: 335-344.

Relative Products

Corticosterone competitive ELISA (TBS31002)
Fast Human IFN-γ ELISA Maxi (TBS3230)
Tribo™ Human CA125 ELISA (TBS3214)
Tribo™ Human CA19-9 ELISA (TBS3215)
Tribo™ Human ß2-microglobulin ELISA (TBS3218)
Protein Cell Lysis Buffer (catalog# TBS5001)
Protein Assay Kit (Catalog# TBS2005)
TMB Substrate System (Catalog# TBS502)

96-wll Plate Layout Sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												