ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Aldosterone

Chemiluminescent Immunoassay Kit

1 Plate Kit Catalog Number K052-C1
5 Plate Kit Catalog Number K052-C5

Species Independent

Sample Types Validated:

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

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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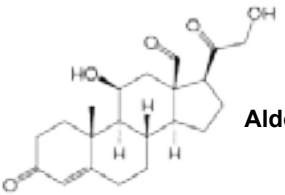
BACKGROUND

Aldosterone, $C_{21}H_{28}O_5$, is a mineralocorticoid first isolated by the husband and wife team of Simpson and Tait at University College, London in 1953[1]. Initially called electrocortin, 21 mg was isolated from 500 kg of beef adrenal glands. Aldosterone controls the sodium-potassium balance through the unidirectional salt reabsorption in a variety of tissues and glands[2,3]. Synthesized from cholesterol in the zona glomerulosa of the adrenal cortex, secretion is regulated through the renin-angiotensin system[4]. 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-glucuronide[5] and this excretion is generally 2-20 µg/24 hour urine collection[6].



Aldosterone

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). 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. Under physiologic conditions, pituitary adrenocorticotropic hormone is not a major factor in regulating aldosterone secretion.

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 reaction. *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.

ASSAY PRINCIPLE

The DetectX® Aldosterone Chemiluminescent Immunoassay (CLIA) kit is designed to quantitatively measure Aldosterone present in extracted serum and plasma, or in saliva, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total aldosterone in extracted serum or plasma and fecal 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. Standards or diluted samples are pipetted into a white microtiter plate coated with an antibody to capture sheep antibodies. An aldosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to aldosterone to each well. After an overnight incubation at 4°C, the plate is washed and substrate is added. The chemiluminescent substrate reacts with the bound aldosterone-peroxidase conjugate to generate light. The generated luminescent signal is detected in a microtiter plate luminometer or multmode reader capable of measuring luminescence. The concentration of the aldosterone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Aldosterone ELISA Kits	K052-H1/H5
Allopregnanolone ELISA Kits	K061-H1/H5
Creatinine Serum Detection Kits	KB02-H1/H2
Creatinine Urinary Detection Kits	K002-H1/H5
Cystatin C Human ELISA Kit	K012-H1
DHEA-S ELISA Kits	K054-H1/H5
Hemoglobin High-Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Retinol Binding Protein Multi-Format ELISA Kits	K062-H1/H5
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5

SUPPLIED COMPONENTS

Coated White 96 Well Plate
A white plastic microplate(s) with 1x8 strips coated with donkey anti-sheep IgG.

Kit K052-C1 **or** -C5	1 **or** 5 Each	Catalog Number X063-1EA

Aldosterone Standard
Aldosterone at 40,000 pg/mL in a special stabilizing solution.

Kit K052-C1 **or** -C5	125 µL **or** 625 µL	Catalog Number C182-125UL **or** -625UL

DetectX® Aldosterone CLIA Antibody
A sheep polyclonal antibody specific for Aldosterone.

Kit K052-C1 **or** -C5	3 mL **or** 13 mL	Catalog Number C184-3ML **or** -13ML

DetectX® Aldosterone CLIA Conjugate
An aldosterone-peroxidase conjugate in a special stabilizing solution.

Kit K052-C1 **or** -C5	3 mL **or** 13 mL	Catalog Number C185-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that must be diluted with deionized or distilled water.

Kit K052-C1 **or** -C5	28 mL **or** 55 mL	Catalog Number X065-28ML **or** -55ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.

Kit K052-C1 **or** -C5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

Substrate Solution A

Kit K052-C1 **or** -C5	6 mL **or** 28 mL	Catalog Number X077-6ML **or** -28ML

Substrate Solution B

Kit K052-C1 **or** -C5	6 mL **or** 28 mL	Catalog Number X078-6ML **or** -28ML

Plate Sealer

Kit K052-C1 **or** -C5	1 **or** 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

Distilled or deionized water.

A Speedvac or other centrifugal vacuum concentrator or a manifold and inert gas supply, such as nitrogen or helium, to evaporate extracted samples.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50, and 100 µL.

A microplate shaker.

96 well microplate reader capable of reading glow chemiluminescence. A list of some models of suitable readers can be found on our website under General Info at www.ArborAssays.com/resources/#general-info. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. **The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:**

> Dilute 5 µL of the Aldosterone Conjugate Concentrate into 245 µL of deionized water. Pipet 5 µL of this dilution into an uncoated white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity of about 0.8 times the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the readers maximum signal.

To properly analyze the data, software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, 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.

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 other manufacturer's Wash Buffers, containing sodium azide will inhibit color production from 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 as prepared on Page 8.

SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, urine samples and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. Aldosterone can be assayed in other sample types by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols.

Aldosterone is identical across all species and we expect this kit may measure aldosterone from sources other than mammalian. The end user should evaluate recoveries of aldosterone in other samples being tested.

SAMPLE PREPARATION

Serum and plasma samples must be extracted with ethyl acetate or similar solvent. Dried fecal samples can be measured as outlined below. Urine samples can be diluted directly in Assay Buffer prior to being run in the assay.

Serum and Plasma Samples

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.

Saliva Samples

Saliva samples should be diluted ≥ 1:2 with the supplied Assay Buffer prior to running in the assay. See our Saliva Sample Handling Instructions at www.ArborAssays.com/assets/saliva-sample-protocol.pdf.

Urine Samples

Urine samples should be diluted ≥ 1:4 with the supplied Assay Buffer prior to running in the assay. Please see our Urinary Creatinine Detection kits, K002-H1 and K002-H5, for assays to measure urine creatinine which can be used to normalize aldosterone in random urine specimens.

Dried Fecal Samples

We have a detailed Extraction Protocol available on our website at:
www.ArborAssays.com/assets/steroid-solid-extraction-protocol.pdf. The ethanol concentration in the final Assay Buffer dilution added to the well should be < 5%.

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. We have validated the assay using RPMI-1640.

Use all Samples within 2 hours of preparation, or stored at ≤ -20°C until assaying.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

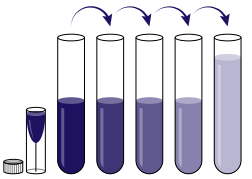
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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.

Standard Preparation

Label test tubes as #1 through #7. Pipet 770 µL of Assay Buffer into tube #1 and 320 µL into tubes #2 to #7. **The aldosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 30 µL of the aldosterone stock solution to tube #1 and vortex completely. Take 160 µL of the aldosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of aldosterone will be 1,500, 500, 166.7, 55.56, 18.52, 6.173 and 2.058 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	770	320	320	320	320	320	320
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	30	160	160	160	160	160	160
Final Conc (pg/mL)	1,500	500	166.7	55.56	18.52	6.173	2.058

Chemiluminescent Substrate

Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine aldosterone concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 100 µL of samples or standards into wells in the plate.
3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Aldosterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Aldosterone Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. 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. We recommend shaking at around 700–900 rpm.
8. Store the sealed plate at 4°C overnight.
9. The following day remove the Chemiluminescent Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**
10. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
11. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.
12. Incubate the plate at room temperature for 5 minutes without shaking.
13. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.
14. Use the plate reader's built-in 4PLC software capabilities to calculate Aldosterone concentration for each sample.

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.

CALCULATION OF RESULTS

Average the duplicate RLU 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 RLU's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-aldosterone-chemiluminescent-clia-kit.assay

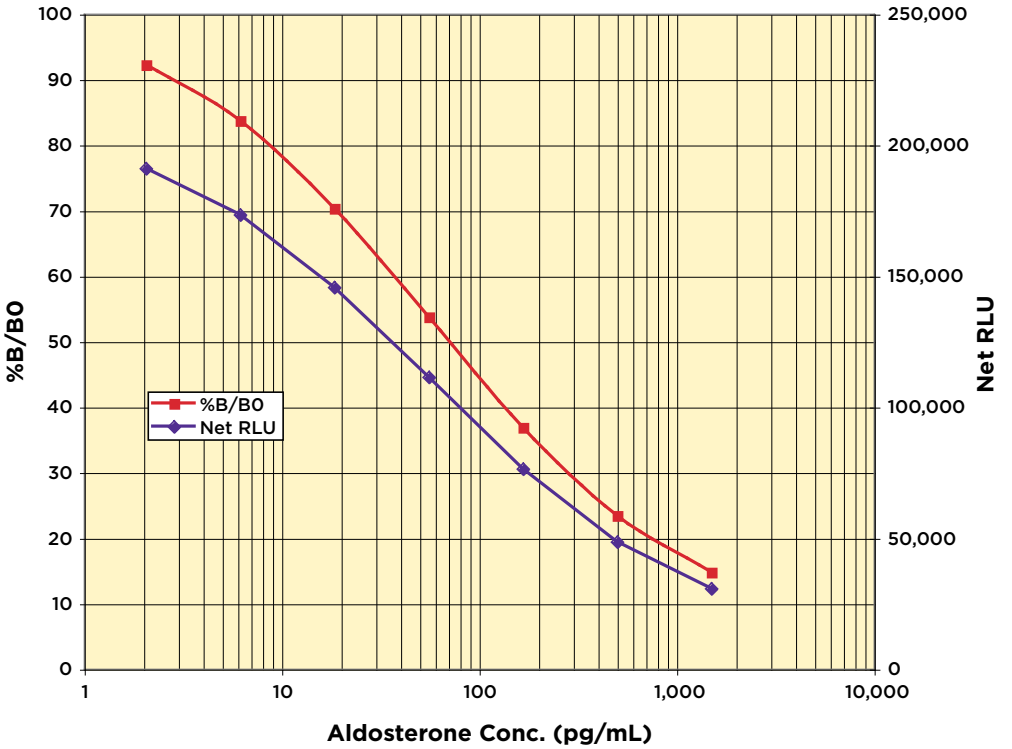
TYPICAL DATA

Sample	Mean RLU	Net RLU	% B/B0	Aldosterone Conc. (pg/mL)
NSB	6,095	0	-	-
Standard 1	36,780	30,685	14.81	1,500
Standard 2	54,665	48,570	23.45	500
Standard 3	82,505	76,410	36.88	166.7
Standard 4	117,475	111,380	53.77	55.56
Standard 5	151,840	145,745	70.35	18.52
Standard 6	179,560	173,465	83.73	6.173
Standard 7	197,235	191,140	92.27	2.058
B0	213,255	207,160	100	0
Sample 1	92,340	86,245	41.63	120.2
Sample 2	150,050	143,955	69.49	19.85

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.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 1.84 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 2.35 pg/mL

Intra Assay Precision

Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Aldosterone concentrations were:

Sample	Aldosterone Conc. (pg/mL)	%CV
1	116.0	5.8
2	21.8	10.1
3	6.33	11.4

Inter Assay Precision

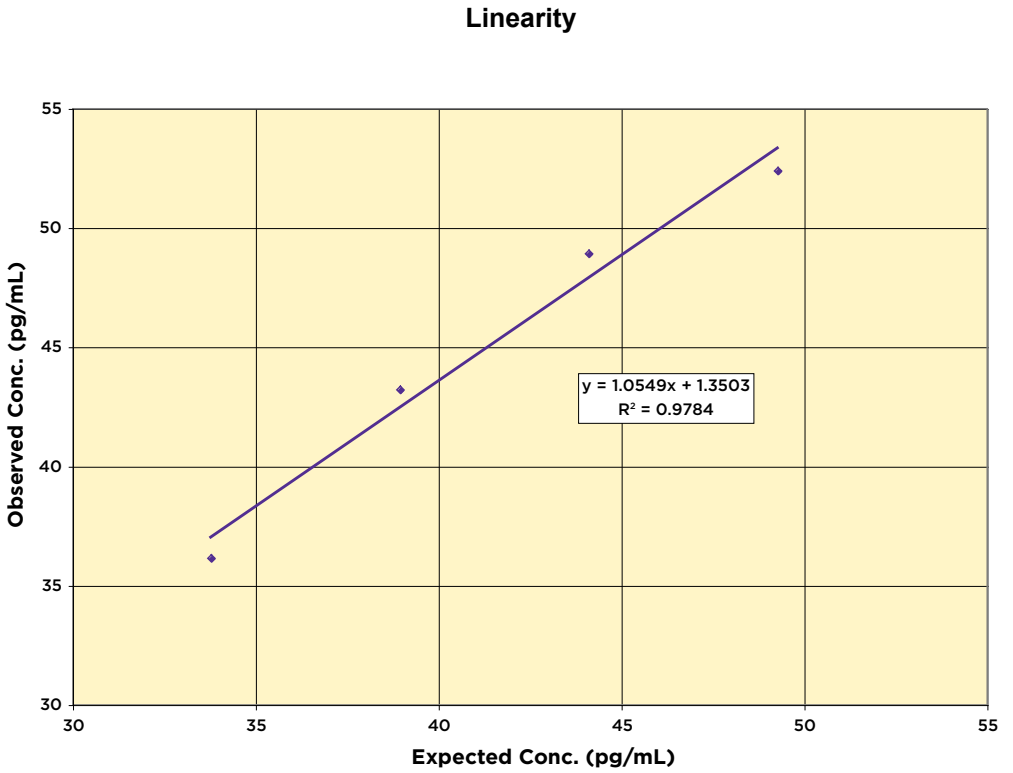
Three urine samples were diluted with Assay Buffer and run in duplicate in twenty-one assays run over multiple days by four operators. The mean and precision of the calculated Aldosterone concentrations were:

Sample	Aldosterone Conc. (pg/mL)	%CV
1	120.2	8.7
2	23.9	13.7
3	6.89	20.0

Linearity

Linearity was determined by taking two urine samples, one with a high diluted aldosterone level of 54.4 pg/mL and one with a lower diluted level of 28.6 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	52.4	49.3	106.3
60%	40%	48.9	44.1	110.9
40%	60%	43.2	39.0	110.9
20%	80%	36.1	33.8	107.0
			Mean Recovery	**108.8%**



SAMPLE VALUES

Four random human serum samples were tested in the CLIA assay. Serum values ranged from 5.59 to 185.4 pg/mL with an average of 66.3 pg/mL. Human urine samples were tested in the assay and the values ranged from 523.2 to 3,216 pg/mL with an average of 1,514.78 pg/mL. One dog urine sample was tested and it read at 6,169 pg/mL.

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%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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