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Datasheet for ABIN2866586
Aldosterone CLIA Kit

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Overview

Quantity:	96 tests
Target:	Aldosterone (ALD)
Reactivity:	Various Species, Human, Dog
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Aldosterone Chemiluminescent Immunoassay (CLIA) kit is designed to quantitative- ly measure Aldosterone present in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Serum, Plasma (EDTA), Plasma (heparin), Urine, Fecal, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Components:	Coated White 96 Well Plates A white plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 Or 5 each Aldosterone Standard Aldosterone at 40,000 pg/mL in a special stabilizing solution. 125 Or 625 µL DetectX® Aldosterone CLIA Antibody A sheep polyclonal antibody specific for Aldosterone. 3 mL Or 13 mL DetectX® Aldosterone CLIA Conjugate An aldosterone-peroxidase conjugate in a special stabilizing solution. 3 mL Or 13 mL

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Product Details

	Assay Buffer Concentrate A 5X concentrate that must be diluted with deionized or distilled water. 28 mL Or 55 mL Wash Buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL Or 125 mL Substrate Solution A 6 mL Or 28 mL Substrate Solution B 6 mL Or 28 mL Plate Sealer 1 Or 5 each
Material not included:	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. 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 de- pendant 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 pro- tocol: 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. 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.

Target Details

Target:	Aldosterone (ALD)
Alternative Name:	Aldosterone (ALD Products)
Target Type:	Hormone
Background:	Aldosterone, C21H28O5, is a mineralocorticoid first isolated by the husband and wife team of Simp- son 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).

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Target Details

	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 (renovas- cular disease, salt depletion, potassium loading, cardiac failure with ascites, pregnancy, Bartter syndrome). The renin-angiotensin system is the primary regulator of the synthesis and secre- tion 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

Application Details

Application Notes:	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 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.
Plate:	Pre-coated
Protocol:	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 sam- ples 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.

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	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 lumines- cence. 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.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine aldosterone concentrations. 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 deion- ized 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 sev- eral 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.
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. Urine

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	Samples Urine samples should be diluted ≥ 1:4 with the supplied Assay Buffer prior 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: The ethanol concen- tration in the final Assay Buffer dilution added to the well should be <5 % .
Assay Procedure:	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 4ºC. 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 wells to act as maximum binding wells (Bo or 0 pg/mL). 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. 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.
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 MyAssays™ online tool

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Application Details

	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.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had labo- ratory 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.
Storage:	4 °C,RT
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

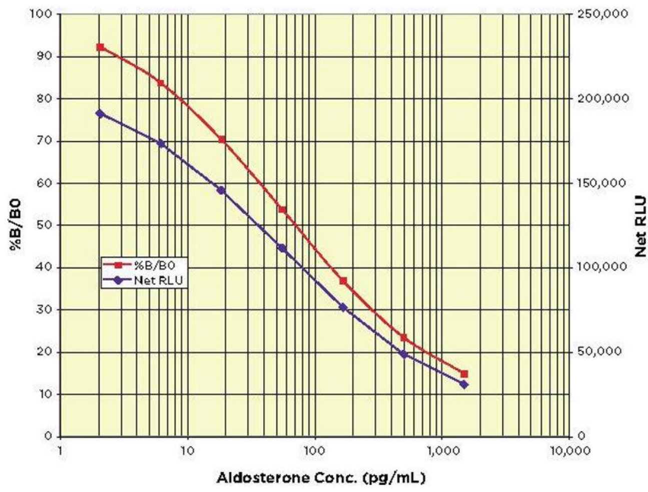

Image 1.