Datasheet for ABIN2815090

Corticosterone CLIA Kit

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Overview

Quantity:	96 tests
Target:	Corticosterone (CORT)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Corticosterone Immunoassay kit is designed to quantitatively measure Corticosterone present in extracted dried fecal samples, serum, plasma and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Fecal, Serum, Plasma (EDTA), Plasma (heparin), Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Cross-Reactivity (Details):	The following cross reactants were tested in the EIA assay and calculated at the 50 % binding point. Steroid Cross Reactivity: Corticosterone 100 %, Desoxycorticosterone 12.30 %, Tetrahydrocorticosterone 0.76 %, Aldosterone 0.62 %, Cortisol 0.38 %, Progesterone 0.24 %, Corticosterone-21-Hemisuccinate < 0.1 %, Cortisone < 0.08 %, Estradiol < 0.08 %
Components:	Coated White 96 Well Plates White plastic break-apart strip microtiter plate(s) coated with donkey anti-sheep IgG. 1 or 5 Each Corticosterone standard Corticosterone at 50,000 pg/mL in a special stabilizing solution. 125 or

Product Details

	625 µL DetectX® Corticosterone CliA Antibody A sheep polyclonal antibody specific for corticosterone. 3 mL or 13 mL DetectX® Corticosterone CliA Conjugate A corticosterone-peroxidase conjugate in a special stabilizing solution. 3 mL or 13 mL Assay buffer Concentrate A 5X concentrate that must be diluted with deionized or distilled water. 28 mL or 55 mL Dissociation reagent 1 mL or 5 mL Dissociation reagent is to be used only with serum and Plasma samples. Wash buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL or 125 mL Substrate solution A 6mL or 28 mL Substrate solution b 6mL or 28 mL Plate sealer 1 or 5 Each
Material not included:	Distilled or deionized water. Microplate shaker. Repeater pipet with disposable tips capable of dispensing 25 µL and 100 µL. 96 well microplate reader capable of reading glow chemiluminescence. A list of some models of suitable readers can be found on our website at www.ArborAssays.com/resources/lit.asp. 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 Corticosterone Conjugate into 995 µL of deionized water. Pipet 5 µL of diluted conjugate into a white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity slightly above the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the 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:	Corticosterone (CORT)
Alternative Name:	Corticosterone (CORT Products)

Target Details

Target Type:	Hormone
Background:	Corticosterone (C H O , Kendall's Compound 'B') is a glucocorticoid secreted by the cortex of 21 30 4 the adrenal gland. Corticosterone is produced in response to stimulation of the adrenal cortex by ACTH and is the precursor of aldosterone. Corticosterone is a major indicator of stress and is the major stress steroid produced in non-human mammals. Studies involving corticosterone and levels of stress include impairment of long term memory retrieval1, chronic corticosterone elevation due to dietary restrictions2 and in response to burn injuries3. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns4,5

Application Details

Application Notes:	This assay has been validated for serum, EDTA and heparin plasma 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. Corticosterone can be assayed in other sample types by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/lit.asp. Corticosterone is identical across all species and we expect this kit may measure corticosterone from sources other than human. The end user should evaluate recoveries of corticosterone in other samples being tested.
Plate:	Pre-coated
Protocol:	This kit measures total corticosterone in serum and plasma and in extracted fecal samples. A corticosterone standard 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. A corticosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a sheep polyclonal antibody to corticosterone to each well. After a two hour incubation the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound corticosterone- peroxidase conjugate to produce light. The generated light is detected in a microtiter plate reader capable of reading luminescence. The concentration of the corticosterone 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 corticosterone 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 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 seven test tubes as #1 through #7. Pipet 470 µL of Assay Buffer into tube #1 and 225 µL into tubes #2 to #7. the corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 30 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 150 µL of the corticosterone 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 corticosterone in tubes 1 through 7 will be 3,000, 1,200, 480, 192, 76.8, 30.72, and 12.288 pg/mL. use all standards within 2 hour of preparation. std 1 std 2 std 3 std 4 std 5 std 6 std 7 Assay buffer (µl) 470 225 225 225 225 225 225 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Vol of Addition (µl) 30 150 150 150 150 150 150 Final Conc (pg/ mL) 3,000 1,200 480 192 76.8 30.72 12.288 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. 1 Plate 2 Plates 3 Plates 4 Plates 5 Plates substrate A & b 5 mL 10 mL 15 mL 20 mL 25 mL Final Mixture 10 mL 20 mL 30 mL 40 mL 50 mL ® 8 EXPECT ASSAY ARTISTRY
Sample Preparation:	Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total corticosterone concentration in serum or plasma. Dissociation reagent is to be used only with serum and Plasma samples. serum and Plasma samples Allow the Dissociation Reagent (DR) to warm completely to room temperature before use. We suggest pipeting 5 µL of DR into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the DR in the tube, vortex gently and incubate at room temperature for 5 minutes or longer. Dilute with

	490 µL of supplied Assay Buffer. This 1:100 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100. note: Dissociation reagent is to be used only with serum and Plasma samples. Dried Fecal samples We have a detailed Extraction Protocol available on our website at: www.ArborAssays.com/resources/lit.asp. The ethanol concentration 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 50 µL of samples or standards into wells in the plate. 3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells. 4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL). 5. Add 25 µL of the DetectX® Corticosterone CLIA Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Corticosterone CLIA 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 2 hours. If the plate is not shaken signals bound will be approximately 45 % lower. 8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels. 9. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet. 10. Incubate the plate at room temperature for 5 minutes without shaking. 11. 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. 12. Use the plate reader's built-in 4PLC software capabilities to calculate corticosterone 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 RLUs 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.
Assay Precision:	Three mouse samples were diluted with Assay Buffer and run in replicates of 20 in an assay. Inter Assay Precision: Three mouse samples were diluted with Assay Buffer and run in duplicates in fifteen assays run over multiple days by three operators.

Application Details

Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide
Precaution of Use:	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 manufacturers' 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.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:	Taves, Mittelstadt, Presman, Hager, Ashwell: "Single-Cell Resolution and Quantitation of Targeted Glucocorticoid Delivery in the Thymus." in: **Cell reports**, Vol. 26, Issue 13, pp. 3629-3642.e4, (2019) (PubMed). Mittelstadt, Taves, Ashwell: "Cutting Edge: De Novo Glucocorticoid Synthesis by Thymic Epithelial Cells Regulates Antigen-Specific Thymocyte Selection." in: **Journal of immunology (Baltimore, Md. : 1950)**, (2018) (PubMed). Li, Munitic, Mittelstadt, Castro, Ashwell: "Suppression of Dendritic Cell-Derived IL-12 by Endogenous Glucocorticoids Is Protective in LPS-Induced Sepsis." in: **PLoS biology**, Vol. 13, Issue 10, pp. e1002269, (2015) (PubMed). Weitnauer, Schmidt, Ng Kuet Leong, Muenchau, Lasitschka, Eckstein, Hübner, Tuckermann, Dalpke: "Bronchial epithelial cells induce alternatively activated dendritic cells dependent on

glucocorticoid receptor signaling." in: **Journal of immunology (Baltimore, Md. : 1950)**, Vol. 193, Issue 3, pp. 1475-84, (2014) (PubMed).

Lambertsen, Gramsbergen, Sivasaravanaparan, Ditzel, Sevelsted-Møller, Oliván-Viguera, Rabjerg, Wulff, Köhler: "Genetic KCa3.1-deficiency produces locomotor hyperactivity and alterations in cerebral monoamine levels." in: **PLoS ONE**, Vol. 7, Issue 10, pp. e47744, (2012) (PubMed).