Datasheet for ABIN577660

Cortisone CLIA Kit

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Overview

Quantity:	96 tests
Target:	Cortisone (COR)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Minimum Detection Limit:	59.6 pg/mL
Application:	ELISA

Product Details

Purpose:	The DetectX® Cortisone Chemiluminescent Immunoassay kit is designed to quantitatively measure Cortisone present in extracted dried fecal samples, urine, saliva, and serum samples.
Brand:	DetectX®
Sample Type:	Serum, Saliva, Urine, Fecal
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Specificity:	Species Independent. Samples Types validated: Dried Fecal Extracts, Urine, Saliva, and Serum
Sensitivity:	10.6 pg/mL
Characteristics:	The Cortisone Chemiluminescent Immunoassay kit is designed to quantitatively measure Cortisone present in samples. A cortisone standard is provided to generate a standard curve for the assay. After a two hour incubation the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound cortisone-peroxidase conjugate to produce light. The generated light is detected in a multilabel microtiter plate reader. Cortisone and cortisol

	concentrations vary due to the activity of two 11ß-hydroxysteroid dehydrogenases (11-HSD). While most tissues have the ability to express either enzyme, 11ß-HSD1 is found primarily in the liver where it converts cortisone to cortisol while 11ß-HSD2 is found in tissues such as the kidney where cortisol receptor binding is required. 11ß-HSD2 deactivates cortisol to cortisone, prohibiting receptor activation. This glucocorticoid 'shuttle' helps to initiate and regulate the anti-inflammatory response. Monitoring the ratio of cortisone:cortisol has applications in diabetes, obesity, metabolic syndrome, osteoporosis, and chronic fatigue syndrome in addition to adrenal diseases. Cortisone and cortisol concentrations exhibit a predictable diurnal pattern and can be measured in extracted dried feces, or in serum, plasma, saliva and urine. A recent publication has suggested that salivary cortisone is a good surrogate marker for serum cortisol.
Components:	Coated White 96 Well Plates A white plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG. 1 Or 5 Each Cortisone Standard Cortisone at 1,000 ng/mL in a special stabilizing solution. 50 µL Or 125 µL DetectX® Cortisone CLIA Antibody A rabbit polyclonal antibody specific for cortisone. 3 mL Or 13 mL DetectX® Cortisone CLIA Conjugate Concentrate A cortisone-peroxidase conjugate concentrate in a special stabilizing solution. 1 mL Or 3.5 mL Conjugate Diluent Contains special stabilizers and additives. 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 NOTE: Dissociation Reagent is to be used only with Serum samples. 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. Repeater pipet with disposable tips capable of dispensing 25 µL 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 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.

Product Details

	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 Cortisone CLIA Conjugate Concentrate 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 close to the maximum binding signal for the assay. Adjust the gain, integration time or sensitivity so that your reader is giving close to its 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:	Cortisone (COR)
Alternative Name:	Cortisone (COR Products)
Target Type:	Hormone
Background:	Cortisone (C21H28O5, Kendall's Compound 'E') was identified by Mason, Myers and Kendall in 1936 as Compound E extracted from bovine suprarenal gland tissue that had the qualitative but not quantitative activity of cortin. The presence of multiple cortin-like compounds led the authors to speculate that the study of Compound E would reveal the nature of cortin1. Compound E is now called cortisone and the more active Compound F, cortisol, and the concentrations of these two glucocorticoids vary due to the activity of two 11ß-hydroxysteroid dehydrogenases (11-HSD) 2,3. While most tissues have the ability to express either enzyme, 11ß-HSD1 is found primarily in the liver where it converts cortisone to cortisol while 11ß-HSD2 is found in tissues such as the kidney where cortisol receptor binding is required. 11ß-HSD2 deactivates cortisol to cortisone, prohibiting receptor activation. This glucocorticoid "shuttle" helps to initiate and regulate the anti-inflam- matory response, making cortisone one of the modern "wonder drugs". Monitoring the ratio of cortisone:cortisol has applications in diabetes, obesity, metabolic syndrome, osteoporosis, and chronic fatigue syndrome in addition to adrenal diseases4-7. Cortisone and cortisol concentrations exhibit a predictable diurnal pattern and can be measured in extracted dried feces, or in serum, plasma, saliva and urine. A recent publication8 has suggested that salivary cortisone is a good surrogate marker for serum cortisol

Application Details

Application Notes:	This assay has been validated for urine, saliva, and serum 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. Cortisone is identical across all species and we expect this kit should measure cortisone from sources other than human. The end user should evaluate recoveries of cortisone in other samples being tested.
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	This kit measures total cortisone in serum and plasma and in extracted fecal samples. A cortisone 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 rabbit antibodies. A cortisone-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 cortisone to each well. After a two hour incubation the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound cortisone-peroxidase conjugate to produce light. The generated light is detected in a microtiter plate reader capable of reading luminescence. The concentration of the cortisone 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 corticos- terone 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 at room temperature for 3 months. Cortisone Conjugate The supplied Cortisone Conjugate Concentrate should be diluted 1:4 with

	the Conjugate Diluent. Once diluted the Cortisone conjugate is stable for one month when stored at 4 °C. Standard Preparation Label nine test tubes as #1 through #9. Pipet 490 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #9. Carefully add 10 µL of the cortisone stock solution to tube #1 and vortex complete- ly. Take 250 µL of the cortisone solution in tube #1 and add it to tube #2 and vortex completely. Re- peat the serial dilutions for tubes #3 through #9. The concentration of cortisone in tubes 1 through 9 will be 20,000, 10,000, 5,000, 2,500, 1,250, 625, 312.5 156.3, and 78.1 pg/mL. Use all Standards within 2 hours of preparation.
Sample Preparation:	Serum samples need to be treated with the supplied Dissociation Reagent. Addition of this re-agent will yield the total cortisone concentration in serum. Dissociation Reagent is to be used only with Serum or Plasma samples. Free cortisone can be measured in saliva and urine samples as directed below. Dried Fecal Samples We have a detailed Extraction Protocol available on our website at: http://www.ArborAssays.com/resources/lit.asp. The ethanol concentration in the final Assay Buf- fer dilution added to the well should be <5 % . Saliva Samples Saliva samples should be frozen and thawed, then centrifuged at 14,000 rpm for 15 minutes. The supernatant should be diluted 1:5 to 1:10 with the supplied Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions at: http://www.arborassays.com/assets/saliva-sample-protocol.pdf. Urine Samples Urine samples should be diluted ≥ 1:100 with the supplied Assay Buffer prior running in the assay. 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.
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 (B0 or 0 pg/mL). 5. Add 25 µL of the DetectX® Cortisone Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Cortisone Antibody to each well, except the NSB wells, using a

Application Details

	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 Cortisone concentration for each sample.
Calculation of Results:	All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU readings for each standard and sample. Cre- ate 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 from http://www.myassays.com/arbor-assays-cortisone-chemiluminescent-clia-kit.assay to calculate the data. QR code for Data Analysis: *The MyAssays logo is a registered trademark of MyAssays Ltd. typical data Sample Mean RLU Net RLU % B/B0 Cortisone Conc. (pg/mL) NSB 7,660 0 - Standard 1 290,990 283,330 22.07 20,000 Standard 2 366,485 358,825 27.95 10,000 Standard 3 443,125 435,465 33.92 5,000 Standard 4 525,230 517,570 40.31 2,500 Standard 5 626,385 618,725 48.19 1,250 Standard 6 755,005 747,345 58.21 625 Standard 7 901,865 894,205 69.65 312.5 Standard 8 1,032,540 1,024,880 79.83 156.25 Standard 9 1,147,060 1,139,400 88.75 78.1 B0 1,291,500 1,283,840 100 0 Sample 1 384,985 377,325 29.39 7,879 Sample 2 947,150 939,490 73.18 237.5 Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 100 pg/mL of cortisone is equivalent to 277.6 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

Handling

	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 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,RT
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:	Kargl, Arshad, Salman, Schurman, Del Corral: "11β-hydroxysteroid dehydrogenase type-II activity is affected by grapefruit juice and intense muscular work." in: **Archives of endocrinology and metabolism**, (2017) (PubMed). Teshima, Matsumoto, Okusa, Uchiyama, Koyama: "Carbenoxolone Disodium Treatment for Canine Pituitary-Dependent Hyperadrenocorticism." in: **PLoS ONE**, Vol. 11, Issue 11, pp. e0166267, (2016) (PubMed).

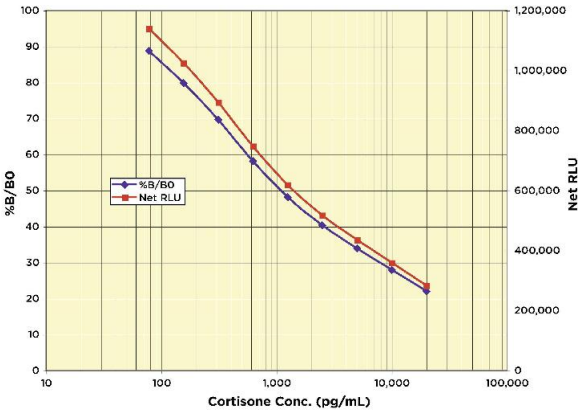

Image 1.

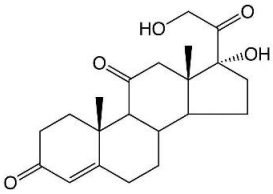

Image 2.