Datasheet for ABIN2866574

Cortisone ELISA Kit

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Overview

Quantity:	96 tests
Target:	Cortisone (COR)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Cortisone Enzyme Immunoassay kit is designed to quantitatively measure cortisone present in extracted dried fecal samples, urine, saliva, plasma, and serum samples.
Brand:	DetectX®
Sample Type:	Fecal, Urine, Saliva, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Coated Clear 96 Well Plates A clear 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. 125 µL Or 625 µL DetectX® Cortisone Antibody A rabbit polyclonal antibody specific for cortisone. 3 mL Or 13 mL DetectX® Cortisone Conjugate A cortisone-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 NOTE: Dissociation Reagent is to be used only with Serum

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	and Plasma samples. Wash Buffer Concentrate A 20X concentrate that must be diluted with deionized or distilled water. 30 mL Or 125 mL TMB Substrate 11 mL Or 55 mL Stop Solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL Or 25 mL Plate Sealer Kit 1 Or 5 each
Material not included:	Distilled or deionized water. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Ethanol or methanol for extraction of dried fecal samples. Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

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, plasma, and serum samples and cortisone is identi- cal across all species. 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.
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 clear microtiter plate coated with an antibody to capture rabbit an- tibodies. 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 TMB substrate is added. The substrate reacts with the bound cortisone-peroxidase conjugate to produce a colored product. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. 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 cortisone 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. Standard Preparation Label test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. Carefully add 50 µL of the cortisone stock solution to tube #1 and vortex completely.

	Take 100 µL of the cortisone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of cortisone in tubes 1 through 6 will be 100,000, 25,000, 6,250, 1,562.5, 390.6, and 97.66 pg/mL. Use all Standards within 2 hours of preparation.
Sample Preparation:	Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total sample cortisone concentration. 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. The ethanol concentration in the final Assay Buffer dilution added to the well should be <2.5 % . Saliva Samples Saliva samples should be collected in a Sarstedt Salivette® Saliva Collection Device or frozen and thawed, then centrifuged at 14,000 rpm for 15 minutes. The supernatant should be diluted ≥ 1:5 with the supplied Assay Buffer prior to running the assay. Urine Samples Urine samples must be diluted > 1:5 with the supplied Assay Buffer prior to running the assay. Due to the levels found in urine, dilutions may need to be > 1:100. 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 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 20 % lower. 8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

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	9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet. 10. Incubate the plate at room temperature for 30 minutes without shaking. 11. Add 50 µL of the Stop Solution to each well, using a repeater pipet. 12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. 13. Use the plate reader's built-in 4PLC software capabilities to calculate cortisone concentration for each sample.
Calculation of Results:	Average the duplicate OD 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 OD'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 Sample Mean OD Net OD % B/B0 Cortisone Conc. (pg/mL) NSB 0.066 0 - Standard 1 0.243 0.177 14.05 100,000 Standard 2 0.372 0.306 24.299 25,000 Standard 3 0.54 0.474 37.62 6,250 Standard 4 0.746 0.68 53.97 1,562.5 Standard 5 0.966 0.9 71.43 390.6 Standard 6 1.167 1.101 87.38 97.66 B0 1.326 1.26 100 0 Sample 1 0.479 0.413 32.81 9,959 Sample 2 0.677 0.611 49.35 2,095 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 laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. The antibody coated plate must 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.

Handling

	The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.
Storage:	4 °C,RT
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Images

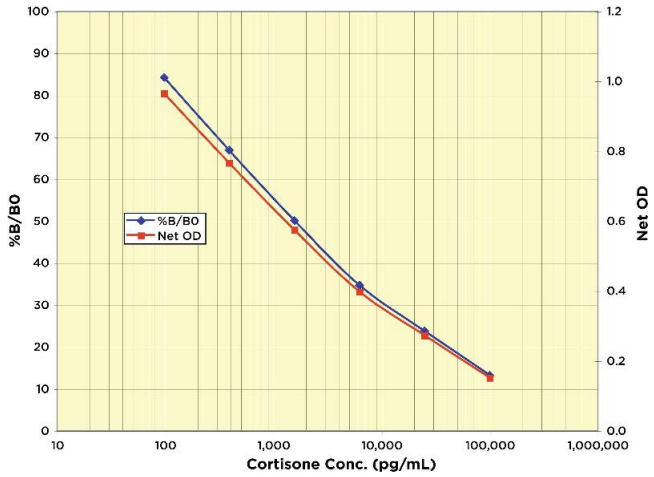

Image 1.

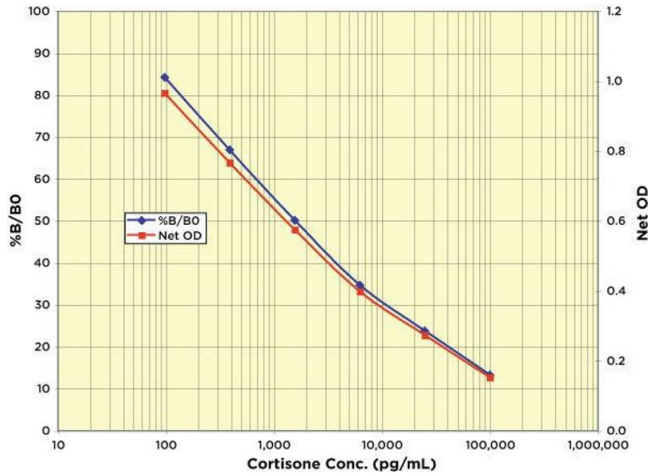

Image 2.