Datasheet for ABIN577640

Cortisol ELISA Kit

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Overview

Quantity:	96 tests
Target:	Cortisol
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Minimum Detection Limit:	45.4 pg/mL
Application:	ELISA

Product Details

Purpose:	The DetectX® Cortisol Immunoassay kit is designed to quantitatively measure cortisol present indried fecal extracts, saliva, urine, serum, plasma and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Fecal, Hair, Plasma (EDTA), Plasma (heparin), Saliva, Serum, Tissue Culture Medium, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Species Independent. Validated samples: Dried Fecal Extracts, Saliva, Urine, Serum, EDTA and Heparin Plasma and Tissue Culture Media
Sensitivity:	17.3 pg/mL
Characteristics:	The Cortisol Immunoassay kit is designed to quantitatively measure cortisol present in dried fecal extracts, saliva, urine, serum, plasma and culture media samples. This kit measures total cortisol in extracted samples, serum and plasma and free cortisol in saliva and urine. A cortisol standard is provided to generate a standard curve for the assay. Standards or diluted samples

	are pipetted into a clear microtiter plate coated with an antibody to capture mouse antibodies. A cortisol-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to cortisol to each well. After an 1 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound cortisol-peroxidase conjugate. After a short incubation the color reaction is read at 450nm. Cortisol is the primary glucocorticoid produced and secreted by the adrenal cortex. It is often referred to as the 'stress hormone' as it is involved in the response to stress and it affects blood pressure, blood sugar levels, and other actions of stress adaptation. Immunologically, cortisol functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression, and disease resistance. In the metabolic aspect, cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Production of cortisol follows an ACTH-dependent circadian rhythm, with a peak level in the morning and decreasing levels throughout the day. Most serum cortisol, all but about 4%, is bound to proteins including corticosteroid binding globulin and serum albumin. Only free cortisol is available to most receptors and it is through these receptors that physiological processes are modulated. Abnormal cortisol levels are being evaluated for correlation with a variety of different conditions, such as prostate cancer, depression, and schizophrenia. It is already known that abnormal levels of cortisol are involved in Cushing's Syndrome and Addison's disease. Calibated - N-Cal Kit, NIST-Calibrated
Components:	Clear Coated 96 Well Plate Each are coated with goat anti-mouse IgG. 1 x 8 Strip Well 1 or 5 each Whole Well Cortisol standard Cortisol at 32,000 pg/mL in a special stabilizing solution. Calibrated to nist srM 921. 125 µL or 625 µL DetectX® Cortisol Antibody A mouse monoclonal antibody specific for cortisol. 3 mL or 13 mL DetectX® Cortisol Conjugate A cortisol-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 Allow to warm completely to room temperature prior to use. 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 TMB substrate 1 1 mL or 55 mL Stop solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL or 25 mL

Product Details

	Plate sealer 1 or 5 Each
Material not included:	Distilled or deionized water. Repeater pipet with disposable tips capable of dispensing 25 µL, 50 µL and 100 µL. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	Cortisol
Alternative Name:	Cortisol Enzyme (Cortisol Products)
Target Type:	Hormone
Background:	Cortisol, C H O , (hydrocortisone, compound F) is the primary glucocorticoid produced and 21 30 5 secreted by the adrenal cortex. It is often referred to as the "stress hormone" as it is involved in the response to stress and it affects blood pressure, blood sugar levels, and other actions of stress adaptation. Immunologically, cortisol functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression, and disease resistance1. In the metabolic aspect, cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization2. Production of cortisol follows an ACTH-dependent circadian rhythm, with a peak level in the morning and decreasing levels throughout the day. Most serum cortisol, all but about 4 % , is bound to proteins including corticosteroid binding globulin and serum albumin1,3. Only free cortisol is available to most receptors and it is through these receptors that physiological processes are modulated. Abnormal cortisol levels are being evaluated for correlation with a variety of different conditions, such as prostate cancer4, depression5, and schizophrenia6. It is already known that abnormal levels of cortisol are involved in Cushing's Syndrome and Addision's disease7

Application Details

Application Notes:	Cortisol is identical across all species and this kit will measure cortisol from sources other than human. The end user should evaluate recoveries of cortisol in other samples. This assay has been validated for saliva, urine, serum and EDTA and heparin plasma and for tissue culture samples. It has been validated for dried fecal extract samples.

Application Details

	Samples containing particulates should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit.
Assay Time:	1.5 h
Plate:	Pre-coated
Protocol:	Total cortisol is measured in extracted samples and in serum and plasma and free cortisol in saliva and urine. A cortisol standard is provided to generate a standard curve for the assay and all samples must be read off a user-generated standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture mouse antibodies. A cortisol-peroxidase conjugate is added to the wells. The binding reaction is initiated by the addition of a monoclonal antibody to cortisol. The immunological reaction occurs between the limiting amount of added anti-cortisol monoclonal antibody, the cortisol antigen in the sample or standard, and the limiting amount of added cortisol-peroxidase conjugate. As the concentration of cortisol in the sample increases, the amount of cortisol-peroxidase conjugate bound decreases causing an decrease in signal, and vice versa. The signal is generated from the cortisol-peroxidase bound to the anti-cortisol antibody which itself is bound to the goat anti-mouse IgG coated plates. Excess cortisol-peroxidase does not bind to the plates and is washed out of the well prior to the addition of substrate. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound cortisol-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader at 450 nm wavelength. The concentration of the cortisol 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. 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 for 3 months at 4 °C. 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.

Application Details

	Standard Preparation Label six test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #6. the cortisol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the cortisol stock solution to tube #1 and vortex completely. Take 250 µL of the cortisol 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 cortisol in tubes 1 through 6 will be 3,200, 1,600, 800, 400, 200, and 100 pg/mL. Use all Standards within 2 hour of preparation. Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 assay Buffer Volume (µL) 450 250 250 250 250 250 addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Volume of addition (µL) 50 250 250 250 250 250 Final Conc (pg/ mL) 3,200 1,600 800 400 200 100 ® 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 cortisol concentration in serum or plasma. Dissociation reagent is to be used only with serum and Plasma samples. Free cortisol 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: 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 % . serum and Plasma samples The normal reference range for human serum cortisol is 2-25 µg/dL (20-250 ng/mL)8. 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 by adding 490 µL of supplied Assay Buffer. This 1:100 dilution can be diluted further with Assay Buffer for higher cortisol sample concentrations. Final serum and plasma dilutions must be ≥ 1:100. notE: Dissociation reagent is to be used only with serum and Plasma samples. saliva samples Saliva samples should be diluted ≥ 1:4 or greater with the supplied Assay Buffer prior 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 ≥ 1:8 with the supplied Assay Buffer prior running in the assay. Urinary cortisol normally ranges from 0.7-119 µg/gram9 of creatinine or approximately 100,000 to 1,000,000 pg/mL9 in 24 hour urine samples. Samples may need to be diluted substantially to read within the standard curve range.
Assay Procedure:	We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine cortisol concentrations.

Application Details

	1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. 2. If you are using the 1 by 8 well strip plate version of the kit, K003-H1 or -H5, determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4ºC. Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells. The use of any wells in the whole plate versions of the kit, K003-H1W and K003-H5W will not allow use of unused parts of that plate in a later assay. 3. Pipet 50 µL of samples or standards into wells in the plate. 4. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells. 5. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL). 6. Add 25 µL of the DetectX® Cortisol Conjugate to each well using a repeater pipet. 7. Add 25 µL of the DetectX® Cortisol Antibody to each well, except the NsB wells, using a repeater pipet. 8. 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 1 hour. 9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels. 10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet. 11. Incubate the plate at room temperature for 30 minutes without shaking. 12. Add 50 µ L of the Stop Solution to each well, using a repeater pipet. 13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. 14. Use the plate reader's built-in 4PLC software capabilities to calculate cortisol 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 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.
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 needs to be stored desiccated.

Handling

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

Publications

Product cited in:	Schmitz, Llorens, Pracht, Thom, Correia, Zafar, Ferrer, Zerr: "Regulation of human cerebrospinal fluid malate dehydrogenase 1 in sporadic Creutzfeldt-Jakob disease patients." in: **Aging**, Vol. 8, Issue 11, pp. 2927-2935, (2017) (PubMed). There are more publications referencing this product on: Product page

Images

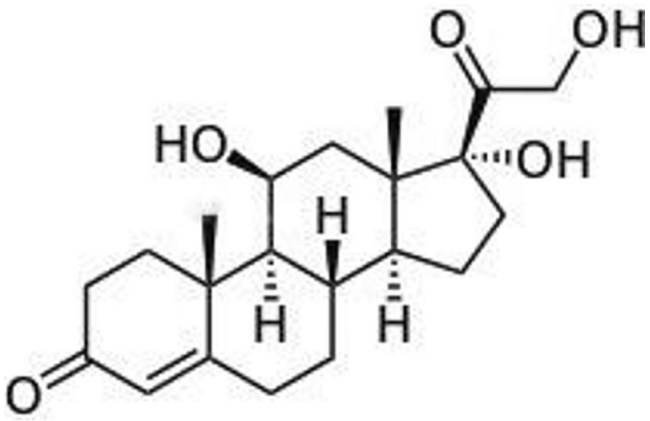

Image 1.

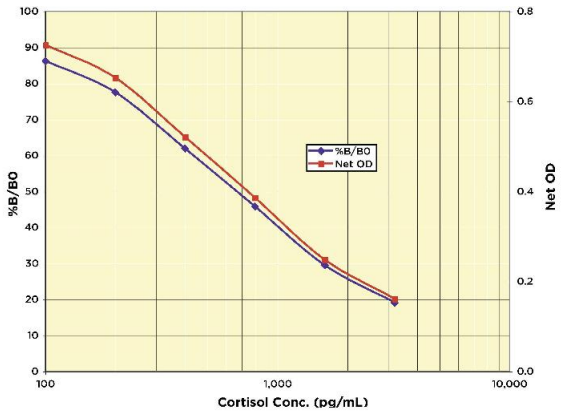
%B/B0
Net OD
Cortisol Conc. (pg/mL)
100
1,000
10,000
0
10
20
30
40
50
60
70
80
90
0.0
0.2
0.4
0.6
0.8

Image 2.