Datasheet for ABIN2866590

Progesterone 17-OH ELISA Kit

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Overview

Quantity:	96 tests
Target:	Progesterone 17-OH
Reactivity:	Various Species, Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® 17-Hydroxyprogesterone Enzyme Immunoassay kit is designed to quantitatively measure 17-Hydroxyprogesterone present in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Fecal, Urine, Serum, Plasma, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Coated Clear 96 Well Plates Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 Or 5 each 17-Hydroxyprogesterone Standard 17-Hydroxyprogesterone at 120,000 pg/mL in a special stabilizing solution. 70 µL Or 350 µL DetectX® 17-Hydroxyprogesterone Antibody A color-coded sheep polyclonal antibody specific for 17-Hydroxyprogesterone. 3 mL Or 13 mL DetectX® 17-Hydroxyprogesterone Conjugate A color-coded 17-Hydroxyprogesterone-peroxidase conjugate in a special stabilizing solution. 3 mL Or 13 mL

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 must be diluted with deionized or distilled water. 30 mL Or 125 mL TMB Substrate Kit 11 mL Or 55 mL Stop Solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL Or 25 mL Plate Sealer 1 Or 5 each
Material not included:	Distilled or deionized water. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Diethyl ether or ethyl acetate for extraction of serum or plasma 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:	Progesterone 17-OH
Alternative Name:	17-Hydroxyprogesterone (Progesterone 17-OH Products)
Target Type:	Hormone
Background:	17-Hydroxyprogesterone, C21H30O3, (4-pregen-17-ol-3, 20-dione, 17HO-P, OHPG) is a steroid hormone from the androgen group and is 0W]ag\bVSaWa found in mammals, reptiles, birds, and other vertebrates. It was first isolated from the adrenal glands of cattle by Pfiffner and North at Park, Davis and Company in Detroit, Michigan in 19401. It is derived from progesterone via 17-hydroxylase, a P450c17 enzyme, or from 17-hydroxypregnenolone via 3ß-hydroxysteroid dehydrogenase 2/?5-4 isomerase. It is primarily produced in the adrenal glands and to some degree in the gonads, specifically the corpus luteum of the ovary. The adrenal glands, ovaries, testes, and placenta produce 17-hydroxyprogesterone. It is hydroxylated at the 11 and 21 position to produce cortisol. Deficiency of either 11- or 21-hydroxylase results in decreased cortisol synthesis, and feedback inhibition of adrenocorticotropic hormone (ACTH) secretion is lost. Consequent increased pituitary release of ACTH will increase production of 17HO-P. But, if 17-alpha-hydroxylase (which allows formation of 17HO-P from progesterone) or 3ß-hydroxysteroid dehydrogenase type 2 (which allows formation of 17-hydroxyprogesterone formation from 17-hydroxypregnenolone) are deficient, 17HO-P levels are low with possible increase in progesterone or pregnenolone respectively. Normal levels are 3-90 ng/dL in children, and in women, 20-100 ng/dL prior to ovulation, and 100-500 ng/dL during the luteal phase2,3.

Target Details

	17-Hydroxyprogesterone Congenital adrenal hyperplasia (CAH) is caused by inherited defects in steroid biosynthesis. The resulting hormone imbalances with reduced glucocorticoids and mineralocorticoids and elevated 17HO-P and androgens can lead to life-threatening, salt-wasting crisis in the newborn and incor- rect gender assignment of virtualized females. Adult-onset CAH may result in hirsutism or infertil- ity in females

Application Details

Application Notes:	This assay has been validated for extracted serum and plasma samples, non-extracted urine, tissue culture samples and dried fecal extracts. Samples containing visible particulate should be centrifuged prior to using. 17HO-P can be assayed in solid sample types or in serum and plasma samples by using one of the extraction protocols. 17HO-P is identical across all species and we expect this kit to measure 17HO-P from all sources. The end user should evaluate recoveries of 17HO-P in other sample matrices being tested.
Plate:	Pre-coated
Protocol:	This kit is not recommended for serum or plasma samples without extraction. The kit will quantitatively measure 17HO-P present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. A 17HO-P 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 sheep antibodies. A 17HO-P-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 17HO-P to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound 17HO-P-peroxidase conjugate. 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 450 nm wavelength. The concentration of the 17HO- P 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 stan- dards and samples be run in duplicate to allow the end user to

	accurately determine 17-Hydroxy- progesterone 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 380 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #6. The 17-Hydroxyprogesterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 20 µL of the 17-Hydroxyproges- terone stock solution to tube #1 and vortex completely. Take 100 µL of the 17HO-P 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 17-Hydroxyprogesterone in tubes 1 through 6 will be 6,000, 2,000, 666.7, 222.2, 74.07 and 24.69 pg/mL. Use all Standards within 2 hours of preparation.
Sample Preparation:	We would recommend the following protocol for serum and plasma. 1. Add diethyl ether or ethyl acetate to serum or plasma samples at a 5:1 (v/v) solvent:sample ratio. 2. Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes. 3. Freeze samples in a dry ice/ethanol bath and pipet off the solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining the solvent solutions. 4. Dry pooled solvent extracts down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20 °C. 5. Redissolve samples at room temperature in the Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement. Dried Fecal Samples: The ethanol concentration in the final Assay Buffer dilution added to the well should be <2.5 % . Urine Samples Urine samples should be diluted at least 1:2 in the provided Assay Buffer. For comparison to crea- tinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection kits, K002-H1 and K002-H5.
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® 17-Hydroxyprogesterone Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® 17-Hydroxyprogesterone 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 1 hour. 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. 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 17-Hydroxyprogesterone 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 online tool typical data Sample Mean OD Net OD % B/B0 17-Hydroxyprogesterone Conc. (pg/mL) NSB 0.062 0 - - Standard 1 0.205 0.143 13.0 6,000 Standard 2 0.347 0.285 25.7 2,000 Standard 3 0.560 0.498 44.9 666.7 Standard 4 0.802 0.740 66.8 222.2 Standard 5 0.998 0.936 84.5 74.07 Standard 6 1.135 1.073 96.8 24.69 B0 1.170 1.108 100 0 Sample 1 0.426 0.364 32.8 1,293.4 Sample 2 0.659 0.597 53.9 425.5 Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 100 pg/mL of 17-Hydroxyprogesterone is equivalent to 302.6 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 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. If the desiccant is pink discard the plate. 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. The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appro- priate 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

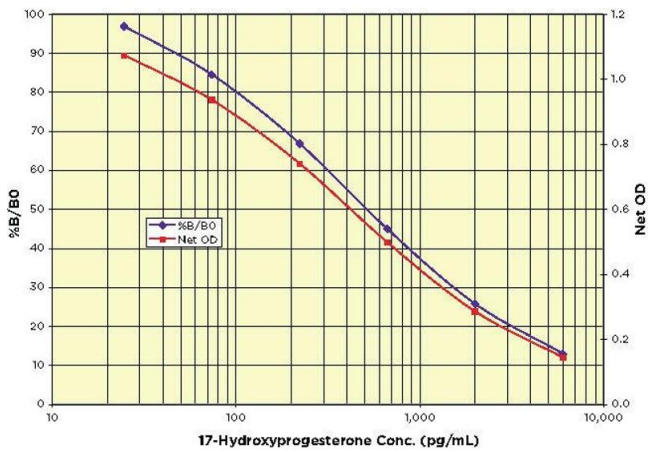

Image 1.