Datasheet for ABIN577680

Progesterone ELISA Kit

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Overview

Quantity:	96 tests
Target:	Progesterone
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Minimum Detection Limit:	52.9 pg/mL
Application:	ELISA

Product Details

Purpose:	The DetectX® Progesterone Immunoassay kit is designed to quantitatively measure Progesteronepresent in extracted dried fecal samples, urine and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Fecal, Urine, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Species Independent. Samples Types validated: Dried Fecal Extracts, Urine and Tissue Culture Media
Sensitivity:	47.9 pg/mL
Characteristics:	The Progesterone Immunoassay kit is designed to quantitatively measure Progesterone present in extracted dried fecal samples, urine and tissue culture media samples. A progesterone 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

Product Details

	mouse antibodies. A progesterone-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 progesterone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound progesterone-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 450nm wavelength.
Components:	Coated Clear 96 Well Plates Clear plastic microtiter plate(s) coated with goat anti-mouse IgG. 1 or 5 Each Progesterone standard Progesterone at 32,000 pg/mL in a special stabilizing solution. 125 or 625 µL DetectX® Progesterone Antibody A mouse monoclonal antibody specific for progesterone. 3 mL or 13 mL DetectX® Progesterone Conjugate A progesterone-peroxidase conjugate in a special stabilizing solution. 3 mL or 13 mL Assay buffer Concentrate A 5X concentrate that should be diluted with deionized or distilled water. 28 or 55 mL Wash buffer Concentrate A 20X concentrate that should 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 1 or 5 Each
Material not included:	Distilled or deionized water. Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL. A microplate shaker. 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:	Progesterone
Abstract:	Progesterone Products
Target Type:	Hormone
Background:	Progesterone, C H O , also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone 21

Target Details

30 2 involved in the female menstrual cycle, gestation and embryogenesis of humans and other species1. Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen2. Progesterone is an essential regulator of human female reproductive function in the uterus, ovary, mammary gland and brain, and plays an important role in non-reproductive tissues such as the cardiovascular system, bone and the central nervous system3. Progesterone action is conveyed by two isoforms of the nuclear progesterone receptor (PR), PRA and PRB. PRA and B are expressed in a variety of normal breast tissue from humans, rats and mice and is also expressed in breast cancer cells4, 5. Progesterone also has neurotrophic roles in the peripheral nervous system as it activates the growth and maturation of axons and stimulates the repair and replacement of myelin sheaths in regenerating nerve fibres6. Progesterone 1. Graham, J. D. and Clarke, C. L., "Physiological action of progesterone in target tissues.", Endocr. Rev., 1997, 18:502-19. 2. Pearlman WH, and Cerceo, E. "The isolation of progesterone from human placenta." J. Biol. Chem., 1952, 278: 73-89. 3. Li, X and O'Malley, BW., "Unfolding the Action of Progesterone Receptors.", J. Biol. Chem., 2003, 278: 39261-39264. 4. Ho, S-M., "Estrogen, Progesterone and Epithelial Ovarian Cancer.", Reprod. Biol. & Endo., 2003, 1:73. 5. Campagnoli, C., Clavel-Chapelon, F., Kaaks, R., Peris, C., and Berrino, F., "Progestins and progesterone in hormone replacement therapy and the risk of breast cancer.", J Steroid Biochem Mol Biol., 2005, 96: 95-108. 6. Koenig, HL, Gong, WH and Pelissier, P., "Role of progesterone in peripheral nerve repair." Revs. of Reprod., 2000, 5:189-199. ® www.ArborAssays.com 3

Application Details

Application Notes:	This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Progesterone can be assayed in other sample types by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols Progesterone is identical across all species and we expect this kit to measure progesterone from all sources. The end user should evaluate recoveries of progesterone in other sample matrices being tested.
Assay Time:	5 h
Plate:	Pre-coated
Protocol:	A progesterone 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 mouse antibodies. A progesterone-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 progesterone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound progesterone-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 450nm wavelength. The concentration of the progesterone 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 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 seven test tubes as #1 through #7. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7. the progesterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the progesterone stock solution to tube #1 and vortex completely. Take 250 µL of the progesterone 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 progesterone in tubes 1 through 7 will be 3,200, 1,600, 800, 400, 200, 100, and 50 pg/mL. Use all standards within 2 hours of preparation.
Sample Preparation:	Dried Fecal samples We have a detailed Extraction Protocol available on our website at: www.arborassays.com/ resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5 % . Urine samples Urine samples should be diluted at least 1:4 times with the provided Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated 2 plate and 10 plate Urinary Creatinine

Application Details

	Detection kits, K002-H1 and K002-H5.
Assay Procedure:	We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine progesterone concentrations. 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® Progesterone Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Progesterone 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 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 progesterone 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.
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:	Dolkart, Amar, Shapira, Marmor, Steinberg, Weinbroum: "Protective effects of rosuvastatin in a rat model of lung contusion: Stimulation of the cyclooxygenase 2-prostaglandin E-2 pathway." in: **Surgery**, Vol. 157, Issue 5, pp. 944-53, (2015) (PubMed). Xiao, Xing, Huo, Fung, Liong, Ching, Xu, Chang, So, Tipoe: "Lycium barbarum polysaccharides therapeutically improve hepatic functions in non-alcoholic steatohepatitis rats and cellular steatosis model." in: **Scientific reports**, Vol. 4, pp. 5587, (2014) (PubMed). Dolkart, E, S, S, P, Aa: "Temporal determination of lung NO system and COX-2 upregulation following ischemia-reperfusion injury." in: **Experimental lung research**, Vol. 40, Issue 1, pp. 22-9, (2014) (PubMed). Zhang, Yu, Kang, Zhu: "Effect of ω-3 fatty acid on gastrointestinal motility after abdominal operation in rats." in: **Mediators of inflammation**, Vol. 2011, pp. 152137, (2011) (PubMed). There are more publications referencing this product on: Product page

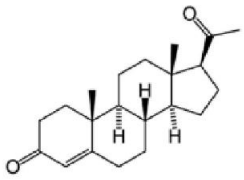

Image 1.

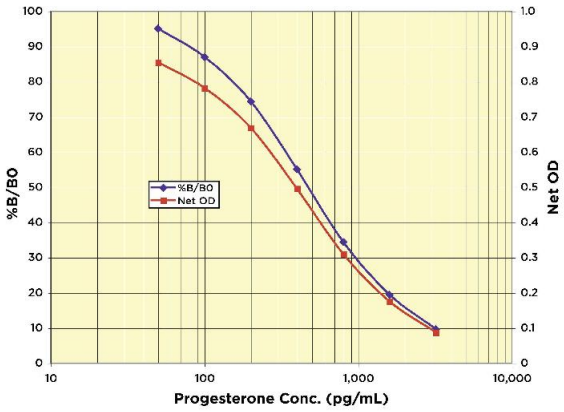

Image 2.

