Datasheet for ABIN2815100

PHGDH ELISA Kit

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Overview

Quantity:	96 tests
Target:	PHGDH
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Pregnanediol-3-Glucuronide (PDG) Immunoassay kit uses a specifically generated antibody to measure PDG and its metabolites in urine and fecal samples, or in extracted serum and plasma.
Brand:	DetectX®
Sample Type:	Fecal, Urine, Serum, Plasma, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	The following cross reactants were tested in the assay and calculated at the 50 % binding point. Steroid Cross Reactivity: Pregnanediol-3-glucuronide 100 %, 20a-hydroxyprogesterone 44.8 %, 20-hydroxyprogesterone 3.16 %, Progesterone 0.2 %, Testosterone 0.2 %, Cortisol 0.06 %, 17-Estradiol 0.04 %
Components:	Coated Clear 96 Well Plates Clear 1 by 8 break-apart strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG. 1 Or 5 Each Pregnanediol-3-Glucuronide (PDG) Standard Pregnanediol-3-Glucuronide (PDG) at 500 ng/mL in

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	a special stabilizing solution. 125 µL Or 625 µL DetectX® Pregnanediol-3-Glucuronide (PDG) Antibody A rabbit polyclonal antibody specific for Pregnanediol-3-Glucuronide. 3 mL Or 13 mL DetectX® Pregnanediol-3-Glucuronide (PDG) Conjugate Pregnanediol-3-Glucuronide-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 with disposable tips capable of dispensing 25 µL, 50 µL 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. Contact your plate reader manufacturer for de- tails.

Target Details

Target:	PHGDH
Alternative Name:	Pregnanediol-3-Glucuronide (PDG) (PHGDH Products)
Background:	Pregnanediol Glucuronide, C27H44O8, also known as PDG (5ß-Pregnnan-3a,20a-diol 3-glucosiduronate) is the major metabolite of progesterone1-4. Progesterone is the hormone involved in the female menstrual cycle, gestation and embryogenesis of humans and other species. Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen5,6. 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 system. 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 cells7,8. Progesterone also has neurotrophic roles in the peripheral nervous system as it activates the

Target Details

	growth and maturation of axons and stimulates the repair and replacement of myelin sheaths in regenerating nerve fibres9. OH Pregnanediol-3-Glucuronide, PDG HO O H HO OH 1. Tait, J.F., Little, B., Tait, S.A.S. et al. Excerpta Medica (Interim Congress Series), 1962, 51:13. 2. Arcos, M., Gurpide, E., Vande Wiele, R.L. and Lieberman, S. Precursors of urinary pregnanediol and their influence on the determination of the secretory production rate of progesterone. J. Clin. Endocrinol., 1964, 24:237-245. 3. Lasley, B.L., Stabenfeldt, G.H., Overstreet, J.W. et al. Urinary hormone levels at the time of ovulation and implantation. Fertil. Steril., 1985, 43:861-867. 4. Stanczyk, F.Z., Miyakawa, I. and Goebelsmann, U. Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. Am J Obstet Gynecol, 1980, 137:443-450. 5. Collins, W.P., Branch, C.M., Collins, P.O. and Sallam H.N. Biochemical indices of the fertile period in women. Int J Fertil., 1981, 26:196-202. 6. Brown, J.8. and Gronow, M. Endocrinology of ovulation prediction, in Clinical Reproductive Endocrinology (R.P. Shearman, Editor). Churchill Livingstone, Edinburgh, 1985, p. 165-184. 7. Wiebe JP. Progesterone metabolites in breast cancer. Endocr Relat Cancer 2006, 13(3):717-738. 8. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. Cancer Epidemiol Biomarkers Prev 2005,14:98-107. 9. 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
Pathways:	Metabolism of Steroid Hormones and Vitamin D, Warburg Effect

Application Details

Application Notes:	This assay has been validated for dried fecal, urine and tissue culture samples. Samples contain- ing visible particulate should be centrifuged prior to using. Pregnanediol-3-glucuronide can be assayed in solid sample types by using one of the extraction protocols lit.asp. Pregnanediol-3-glucuronide (PDG) is identical across all species and we expect this kit to measure pregnanediol-3-glucuronide from all sources. The end user should evaluate recoveries of PDG in other sample matrices being tested.
Plate:	Pre-coated
Protocol:	This kit is not recommended for serum, plasma, or saliva samples without extraction. The kit will quantitatively measure PDG present in diluted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. A PDG standard is provided to generate a standard curve for the assay and all samples should

	be read off the stan- dard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. A PDG-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 PDG to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound PDG-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 PDG 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 preg- nanediol-3-glucuronide 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 eight test tubes as #1 through #8. Pipet 450 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #8. The Pregnanediol-3-Glucuronide stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the preg- nanediol-3-glucuronide stock solution to tube #1 and vortex completely. Take 200 µL of the preg- nanediol-3-glucuronide solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of pregnanediol-3-glucuronide in tubes 1 through 8 will be 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.391 ng/mL. Use all Standards within 2 hours of preparation.
Sample Preparation:	Serum and Plasma Samples. We would recommend the following protocol for serum and

	plasma. 1. Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio. 2. Mix solutions by vortexing for 2 minutes. Allow ether layer to separate for 5 minutes. 3. Freeze samples in a dry ice/ethanol bath and pipet off the ether solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions. 4. Dry pooled ether samples 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 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 Buffer dilution added to the well should be ≤1 % . Urine Samples Urine samples should be diluted at least 1:5 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 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. We suggest you run samples and standards vertically down the plate columns so that unused wells can be easily kept for further experiments. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Ensure the desiccant is blue. Seal the ziploc plate bag and store at 4ºC. 2. Pipet 50 µL of samples or standards into wells in the plate. 3. Pipet 50 µL of Assay Buffer into maximum binding wells (B0 or 0 pg/mL). 4. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells. 5. Add 25 µL of the DetectX® Pregnanediol-3-Glucuronide Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Pregnanediol-3-Glucuronide 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 35 % 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 pregnanediol-3- glucuronide concentration for each sample. NOTE: If

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	you have any unused wells left, please retain the plate frame to holds these wells for the next experiment.
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 from www.myassays.com/arbor-assays-pregnanediol-3-glucuronide-(pdg)-eia-kit.assay to calculate the data. *The MyAssays logo is a registered trademark of MyAssays Ltd. typical data Sample Mean OD Net OD % B/B0 PDG Conc. (ng/mL) NSB 0.048 0 - - Standard 1 0.355 0.307 13.2 50 Standard 2 0.555 0.507 21.8 25 Standard 3 0.794 0.746 32.0 12.5 Standard 4 1.106 1.058 45.4 6.25 Standard 5 1.391 1.343 57.6 3.125 Standard 6 1.706 1.658 71.1 1.563 Standard 7 1.958 1.910 81.9 0.781 Standard 8 2.129 2.081 89.3 0.391 B0 2.379 2.331 100 0 Sample 1 1.307 1.259 54.0 3.9 Sample 2 1.766 1.718 73.7 1.3 Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 100 pg/mL of PDG is equivalent to 201.4 pM.
Assay Precision:	Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay. Inter Assay Precision: Three urine samples were diluted with Assay Buffer and run in duplicates in fourteen assays run over multiple days by three operators.
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide
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. This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' Wash Buffers, containing sodium azide will inhibit color production from the enzyme.

Handling

	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 appro- priate 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.

Images

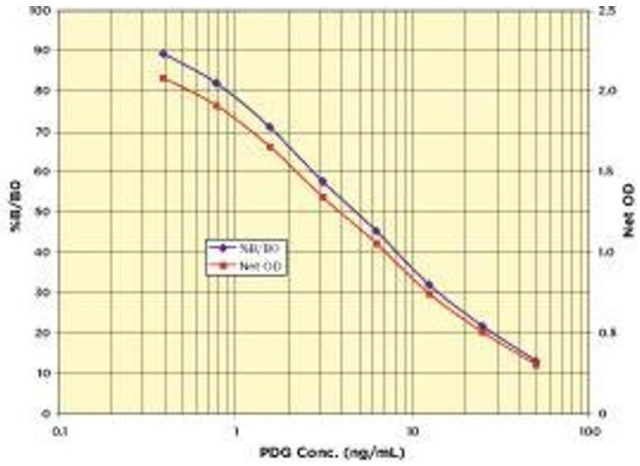

Image 1.