



ARBOR
ASSAYS

DetectX[®]

Pregnanediol-3-Glucuronide (PDG)

Enzyme Immunoassay Kit

1 Plate Kit

Catalog Number K037-H1

5 Plate Kit

Catalog Number K037-H5

SPECIES INDEPENDENT



Sample Types Validated:

**Dried Fecal Extracts, Urine,
Extracted Serum/Plasma, and
Tissue Culture Media**

**Please read this insert completely prior to using the
product.**

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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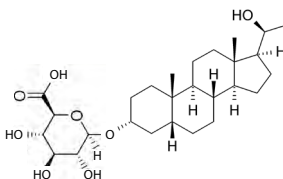
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BACKGROUND

Pregnanediol Glucuronide, $C_{27}H_{44}O_8$, also known as PDG (5 β -Pregnnan-3 α ,20 α -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 progestogen^{5,6}. 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 cells^{7,8}. 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 fibres⁹.

Pregnanediol-3-Glucuronide, PDG



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ASSAY PRINCIPLE

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. 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 standard 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.

RELATED PRODUCTS

KITS

Urinary Creatinine Detection Kit (2 or 10 Plates)	Catalog Number K002-H1/H5
Progesterone Enzyme Immunoassay Kits	Catalog Number K025-H1/H5
Ceruloplasmin Colorimetric Activity Kit	Catalog Number K035-H1
17 β -Estradiol Enzyme Immunoassay Kits	Catalog Number K030-H1/H5
Estrone Enzyme Immunoassay Kits	Catalog Number K031-H1/H5
PGFM (13,14-Dihydro-15-keto-PGF _{alpha}) EIA Kits	Catalog Number K022-H1/H5
Estrone-3-Glucuronide (E1G) EIA Kits	Catalog Number K036-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

Clear 1 by 8 break-apart strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG.
Kit K037-H1 **OR** -H5 1 **OR** 5 Each Catalog Number X016-1EA

Pregnanediol-3-Glucuronide (PDG) Standard

Pregnanediol-3-Glucuronide (PDG) at 500 ng/mL in a special stabilizing solution.
Kit K037-H1 **OR** -H5 125 µL **OR** 625 µL Catalog Number C128-125UL **OR** -625UL

DetectX® Pregnanediol-3-Glucuronide (PDG) Antibody

A rabbit polyclonal antibody specific for Pregnanediol-3-Glucuronide.
Kit K037-H1 **OR** -H5 3 mL **OR** 13 mL Catalog Number C126-3ML **OR** -13ML

DetectX® Pregnanediol-3-Glucuronide (PDG) Conjugate

Pregnanediol-3-Glucuronide-peroxidase conjugate in a special stabilizing solution.
Kit K037-H1 **OR** -H5 3 mL **OR** 13 mL Catalog Number C127-3ML **OR** -13ML

Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.
Kit K037-H1 **OR** -H5 28 **OR** 55 mL Catalog Number X065-28ML **OR** -55ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.
Kit K037-H1 **OR** -H5 30 mL **OR** 125 mL Catalog Number X007-30ML **OR** -125ML

TMB Substrate

Kit K037-H1 **OR** -H5 11 mL **OR** 55 mL Catalog Number X019-11ML **OR** -55ML

Stop Solution

A 1M solution of hydrochloric acid. CAUSTIC.
Kit K037-H1 **OR** -H5 5 mL **OR** 25 mL Catalog Number X020-5ML **OR** -25ML

Plate Sealer

Kit K037-H1 **OR** -H5 1 **OR** 5 Each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25 μ L, 50 μ L and 100 μ L.

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 details.

PRECAUTIONS

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. 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.



SAMPLE TYPES

This assay has been validated for dried fecal, urine and tissue culture samples. Samples containing 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 available on our website at: <http://www.ArborAssays.com/resources/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.

SAMPLE PREPARATION

Serum and Plasma Samples

We have 3 detailed Extraction Protocols available on our website at: <http://www.ArborAssays.com/resources/lit.asp> as a PDF file entitled "Steroid Serum/Plasma Extraction Protocol". 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.

Tissue Culture Media

For measuring pregnanediol-3-glucuronide in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

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 pregnanediol-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 deionized water. Once diluted this is stable at 4°C for 3 months.

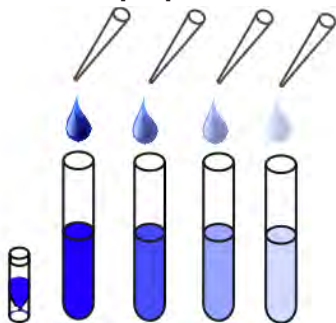
Wash Buffer

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 eight test tubes as #1 through #8. Pipet 450 μ L of Assay Buffer into tube #1 and 200 μ L 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 μ L of the pregnanediol-3-glucuronide stock solution to tube #1 and vortex completely. Take 200 μ L of the pregnanediol-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.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (μL)	450	200	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 5	Std 6
Vol of Addition (μL)	50	200	200	200	200	200	200	200
Final Conc (ng/mL)	50	25	12.5	6.25	3.125	1.563	0.781	0.391

ASSAY PROTOCOL

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 4 °C.
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 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.

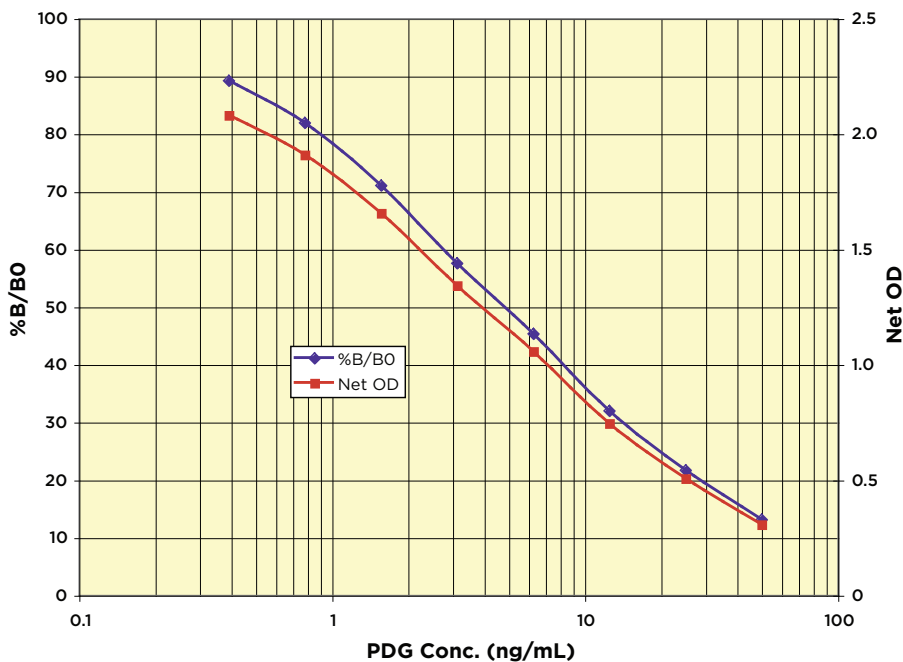
TYPICAL DATA

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.

Typical Standard Curves



**Always run your own standard curves for calculation of results.
Do not use this data.**

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 0.180 ng/mL.

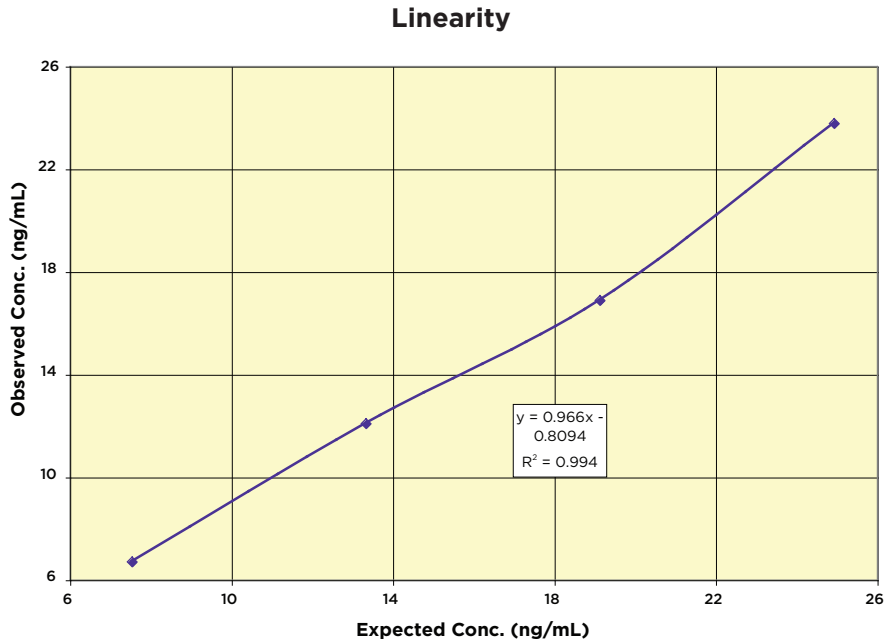
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human urine sample.

Limit of Detection was determined as 0.320 ng/mL

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted pregnanediol-3-glucuronide (PDG) level of 1.70 ng/mL and one with a higher diluted level of 30.7 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Observed Conc. (ng/mL)	Expected Conc. (ng/mL)	% Recovery
80%	20%	23.8	24.9	95.4
60%	40%	16.9	19.1	88.3
40%	60%	12.1	13.3	90.7
20%	80%	6.7	7.5	89.1
Mean Recovery				90.9%



Intra Assay Precision

Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated pregnanediol-3-glucuronide (PDG) concentrations were:

Sample	PDG Conc. (ng/mL)	%CV
1	12.5	2.9
2	4.0	3.7
3	1.5	5.7

Inter Assay Precision

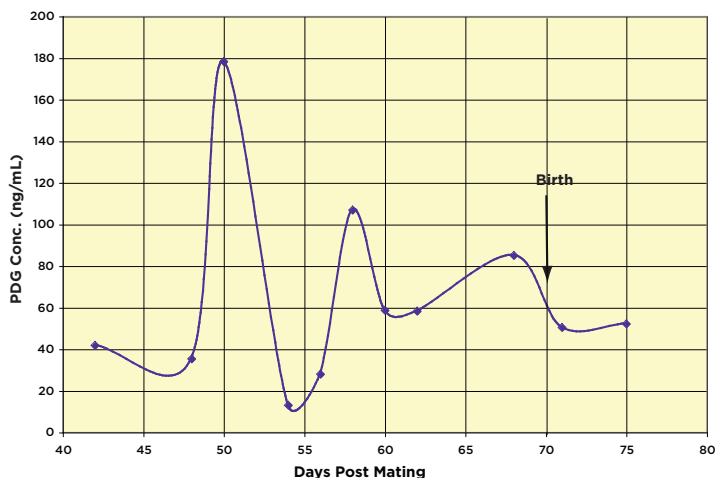
Three urine samples were diluted with Assay Buffer and run in duplicates in fourteen assays run over multiple days by three operators. The mean and precision of the calculated pregnanediol-3-glucuronide (PDG) concentrations were:

Sample	PDG Conc. (ng/mL)	%CV
1	12.3	6.4
2	3.9	5.2
3	1.3	7.5

SAMPLE VALUES

Eleven urine samples from various species were tested in the assay. Adjusted neat concentrations of pregnanediol-3-Glucuronide (PDG) ranged from undetectable to over 1,000 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine detection kit, K002-H1, the values ranged from 19.51 to 784.3 ng/mg creatinine.

Fecal samples from Camarina, a female Iberian Lynx, were extracted and tested in the assay.



The Iberian Lynx samples were the kind gift from Dr. Martin Dehnhard, Leibniz Institute for Zoo and Wildlife Research, Berlin.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Pregnanediol-3-glucuronide	100%
20 α -hydroxyprogesterone	44.8%
20 β -hydroxyprogesterone	3.16%
Progesterone	0.2%
Testosterone	0.2%
Cortisol	0.06%
17 β -Estradiol	0.04%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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