ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Progesterone Metabolites
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K068-H1
5 Plate Kit Catalog Number K068-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts and Urine

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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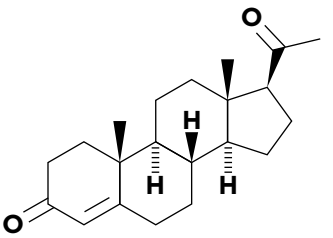
TABLE OF CONTENTS

BACKGROUND

Progesterone, $C_{21}H_{30}O_2$, also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone involved in the female menstrual cycle, gestation and embryogenesis of humans and other species[1]. Progesterone belongs to a class of hormones called progestogens and is the major naturally occurring human progestogen[2]. 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[3].

In different animal species, progesterone can be metabolized and excreted as a variety of general progesterone molecules[4]. A few examples would be fecal 5-reduced progesterone (pregnane) metabolites, pregnanolones and hydroprogesterones. Measurement of these general progesterone molecules can provide vital data about endangered species to aid reproductive strategies. A group specific antibody with a high cross reactivity to most progesterone metabolites would provide strong evidence for an ongoing reproductive cycle [5,6].

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. Schwarzenberger, F., Tomášová, K., Holečková, D., Matern, B., and Möstl, E., "Measurement of Fecal Steroids in the Black Rhinoceros (*Diceros bicornis*) Using Group-Specific Enzyme Immunoassays for 20-Oxo-Pregnanes.", Zoo Biology, 1996; 15:159-171.
5. Kancheva, R., Hill, M., Cibula, D., Včeláková, H., Kancheva, L., Vrbíková, J., Fait, T., Pařízek, A., and Stárka, L., "Relationship of circulating pregnanolone isomers and their polar conjugates to the status of sex, menstrual cycle, and pregnancy.", J. Endocrinology, 2007; 195(1):67-78.
6. Palme, R., Möstl, E., Schellander, K., Bamberg, E., "Faecal Metabolites of Infused ^{14}C-Progesterone in Domestic Livestock.", Reprod Dom Anim, 1997; 32(4): 199-206.

ASSAY PRINCIPLE

The DetectX® Progesterone Metabolite Immunoassay Kit is designed to quantitatively measure Progesterone metabolites present in extracted dried fecal samples and urine. Please read the complete kit insert before performing this assay. 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 rabbit 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 polyclonal antibody to progesterone metabolites to each well. After a 1 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 450 nm 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.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone EIA Kits	K053-H1/H5
Aldosterone ELISA and Chemiluminescent ELISA Kits	K052-H1/H5, K052-C1/C5
Allopregnanolone ELISA Kits, Monoclonal Antibody Based	K061-H1/H5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Corticosterone ELISA and Chemiluminescent ELISA Kits	K014-H1/H5, K014-C1/C5
Cortisol ELISA Kits (Strip Well and Whole Plate)	K003-H1/H5, K003-H1W/H5W
Dehydroepiandrosterone sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Epiandrosterone ELISA Kits	K063-H1/H5
Estradiol ELISA Kits (Non-Invasive and Serum)	K030-H1/H5, KB30-H1/H5
Estriol ELISA Kits	K064-H1/H5
Estrone ELISA Kits	K031-H1/H5
Estrone-3-Glucuronide (E1G) ELISA Kits	K036-H1/H5
Estrone-3-Sulfate (E1S) ELISA Kits	K038-H1/H5
Levonorgestrel (LNG) ELISA Kits	K058-H1/H5
Oxytocin ELISA and Chemiluminescent ELISA Kits	K048-H1/H5, K048-C1/C5
PGFM ELISA Kits	K022-H1/H5
Pregnanediol-3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Prolactin (PRL) ELISA Kit	K040-H1/H5
Testosterone ELISA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.

Kit K068-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

Progesterone Standard
Progesterone at 1,000 ng/mL in a special stabilizing solution.

Kit K068-H1 **or** -H5	40 **or** 200 µL	Catalog Number C252-40UL **or** -200UL

DetectX® Progesterone Antibody
A rabbit polyclonal antibody specific for progesterone metabolites.

Kit K068-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C250-3ML **or** -13ML

DetectX® Progesterone Conjugate
A progesterone-peroxidase conjugate in a special stabilizing solution.

Kit K068-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C251-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.

Kit K068-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 K068-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K068-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

Stop Solution
A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K068-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K068-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, 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. 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 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, however excreted metabolites may vary species to species. The end user should evaluate recoveries of progesterone metabolites in other sample matrices being tested.

SAMPLE PREPARATION

Dried Fecal Samples

We have a detailed Solid Steroid 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 must be < 5%.

Urine Samples

Urine samples must be diluted > 1:8 with the diluted 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.

Use all samples within 2 hours of preparation.

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

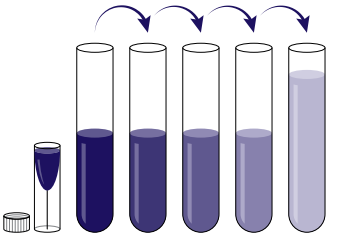
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 six test tubes as #1 through #6. Pipet 990 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. **The progesterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of the progesterone stock solution to tube #1 and vortex completely. Take 200 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 #6. The concentration of progesterone in tubes 1 through 6 will be 10,000, 4,000, 1,600, 640, 256, and 102.4 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**990**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**10**	200	200	200	200	200
Final Conc (pg/mL)	10,000	4,000	1,600	640	256	102.4

ASSAY PROTOCOL

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 4˚C.
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 the maximum binding (B0 or Zero standard) wells.
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 1 hour. If the plate is not shaken signals bound will be approximately 25% 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.

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.

Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-detectx-progesterone-metabolites.assay

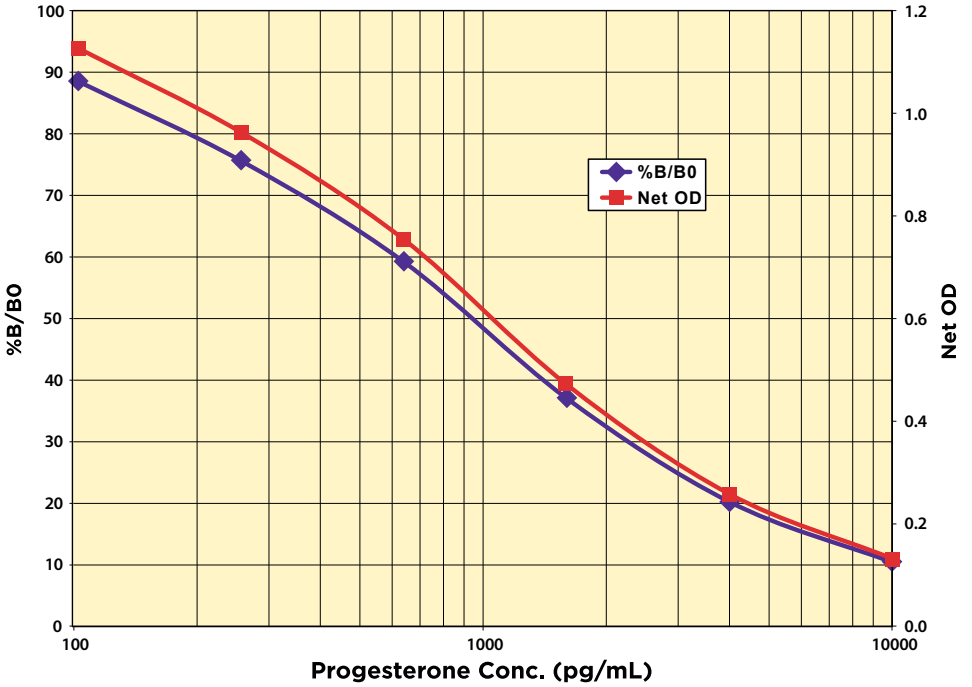
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Progesterone Conc. (pg/mL)
NSB	0.078	-	-	-
Standard 1	0.209	0.132	10.3	10,000
Standard 2	0.335	0.258	20.2	4,000
Standard 3	0.550	0.472	37.1	1,600
Standard 4	0.832	0.755	59.3	640
Standard 5	1.041	0.964	75.7	256
Standard 6	1.206	1.128	88.6	102.4
B0	1.351	1.273	100	0
Sample 1	0.450	0.373	29.3	2,373
Sample 2	0.534	0.457	35.9	1,716

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

Conversion Factor: 100 pg/mL of progesterone is equivalent to 318.0 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

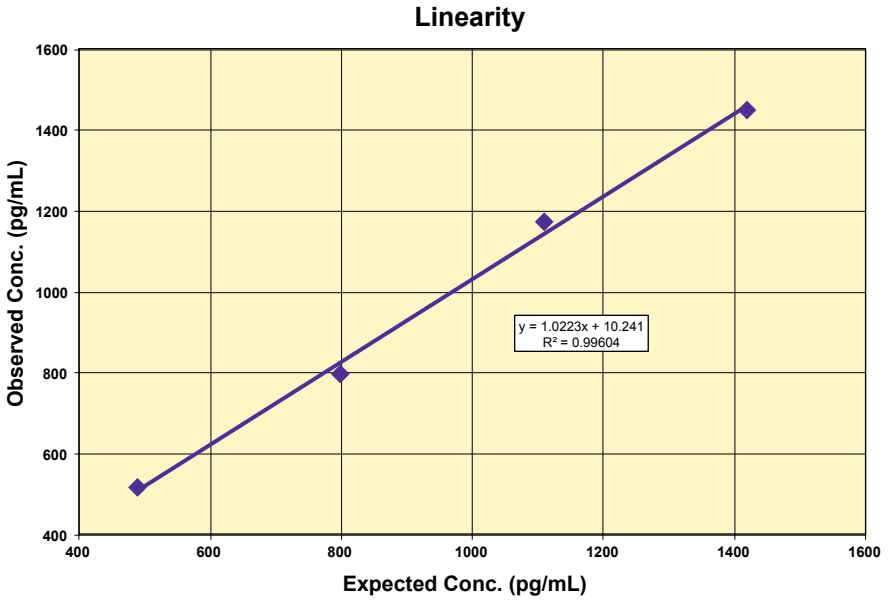
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 51.2 pg/mL.**

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 sample. **Limit of Detection was determined as 57.0 pg/mL**

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted progesterone level of 179.2 pg/mL and one with a higher diluted level of 1,729 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Urine	High Urine	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	489.1	519.9	106.3
60%	40%	799.1	797.5	99.8
40%	60%	1,109	1174	105.9
20%	80%	1,419	1451	102.2
			Mean Recovery	**103.6%**



Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Progesterone concentrations were:

Sample	Progesterone Conc. (pg/mL)	%CV
1	2,377	4.9
2	1,517	7.2
3	710.3	8.0

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in nineteen assays run over multiple days by six operators. The mean and precision of the calculated Progesterone concentrations were:

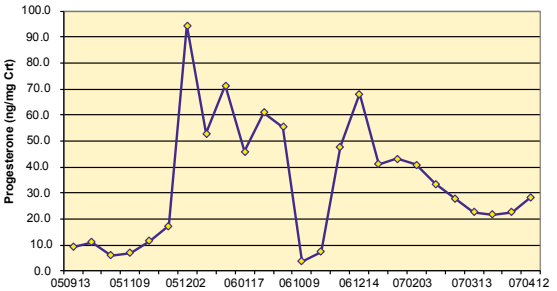
Sample	Progesterone Conc. (pg/mL)	%CV
1	2,442	6.8
2	1,665	6.6
3	844.6	8.8

SAMPLE VALUES

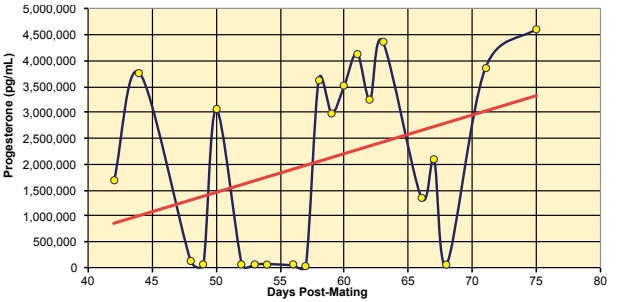
Four human urine samples were tested in the assay, three came from pregnant women who were 17, 21, and 26 weeks pregnant. Adjusted neat concentrations of progesterone ranged from 39.46 to 4312.6 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine detection kit, K002-H1, the values ranged from 49.7 to 215.9 ng/mg creatinine.

Timed urine samples from a pregnant Maned Wolf over a 12 month period, and dried fecal samples from an Iberian Lynx were tested in the assay.

Maned Wolf Urine



Iberian Lynx



Maned wolf samples were the kind gift of Rachel Santymire from Lincoln Park Zoo, Chicago and the Iberian lynx samples were from Martin Dehnhard, Leibniz Institute for Zoo & Wildlife Research, Berlin.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Progesterone	100
5β-dihydroprogesterone	61.9
5α-dihydroprogesterone	56.7
Pregnanolone (5β-Pregnan-3α-ol-20-one)	41.2
Epiallopregnanolone (5α-pregnan-3β-ol-20-one)	38.3
Allopregnanolone	27.3
Pregnenolone	17.6
Epipregnanolone	10.2
17α-hydroxyprogesterone	5.7
11α-hydroxyprogesterone	4.9
20α-hydroxyprogesterone	0.34
Allopregnandiol	0.29

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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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12