ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Progesterone
Enzyme Immunoassay Kit

1 or 5 Strip Plates Catalog Numbers K025-H1/H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, Urine and Tissue Culture Media

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

www.ArborAssays.com

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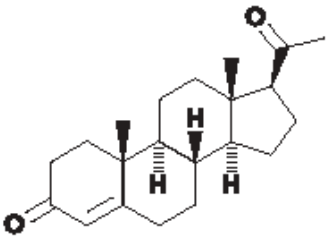
BACKGROUND

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 progestogen and is the major naturally occurring human progestogen[2]. Progesterone, an essential regulator of human female reproductive function in the uterus, ovary, mammary gland and brain, also plays an important role in non-reproductive tissues such as the cardiovascular system, bone and the central nervous system[3]. 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[4, 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 fibres[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. 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.

ASSAY PRINCIPLE

The DetectX® Progesterone Immunoassay Kit is designed to quantitatively measure Progesterone present in extracted dried fecal samples, urine and tissue culture media samples. 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 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 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.
Corticosterone Chemiluminescent Immunoassay Kits	K014-C1/C5
Corticosterone Enzyme Immunoassay Kits	K014-H1/H5
Cortisol Enzyme Immunoassay Kits	K003-H1/H5
Cortisone Chemiluminescent Immunoassay Kits	K017-C1/C5
Cortisone Enzyme Immunoassay Kits	K017-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

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

Kit K025-H1 **or** -H5	1 **or** 5 Each	Catalog Number X012-1EA, 1 x 8 Strip Well

Progesterone Standard
Progesterone at 32,000 pg/mL in a special stabilizing solution.

Kit K025-H1 **or** -H5	125 **or** 625 µL	Catalog Number C092-125UL **or** -625UL

DetectX® Progesterone Antibody
A mouse monoclonal antibody specific for progesterone.

Kit K025-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C090-3ML **or** -13ML

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

Kit K025-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C091-3ML **or** -13ML

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

Kit K025-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 K025-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K025-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K025-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K025-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 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.

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 4-fold with 1X 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 progesterone 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.

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

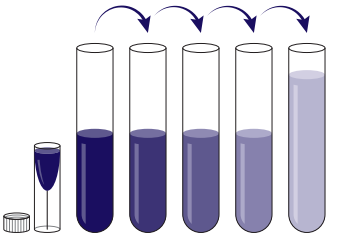
Dilute Assay Buffer Concentrate 5-fold by adding one part of the concentrate to four parts of deionized water. The 1X Assay Buffer is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 20-fold by adding one part of the concentrate to nineteen parts of deionized water. The 1X Wash Buffer is stable at room temperature for 3 months.

Standard Preparation

Label seven test tubes as #1 through #7. Pipet 450 µL of 1X 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.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
1X Assay Buffer (µL)	**450**	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**50**	250	250	250	250	250	250
Final Conc (pg/mL)	3,200	1,600	800	400	200	100	50

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.
2. 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.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL of samples or standards into wells in the plate.
4. Pipet 75 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 50 µL of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the DetectX® Progesterone Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Progesterone Antibody to each well, **except the NSB wells**, using a repeater pipet.
8. 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. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 45% lower.
9. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. 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:
www.myassays.com/arbor-assays-progesterone-eia-kit.assay

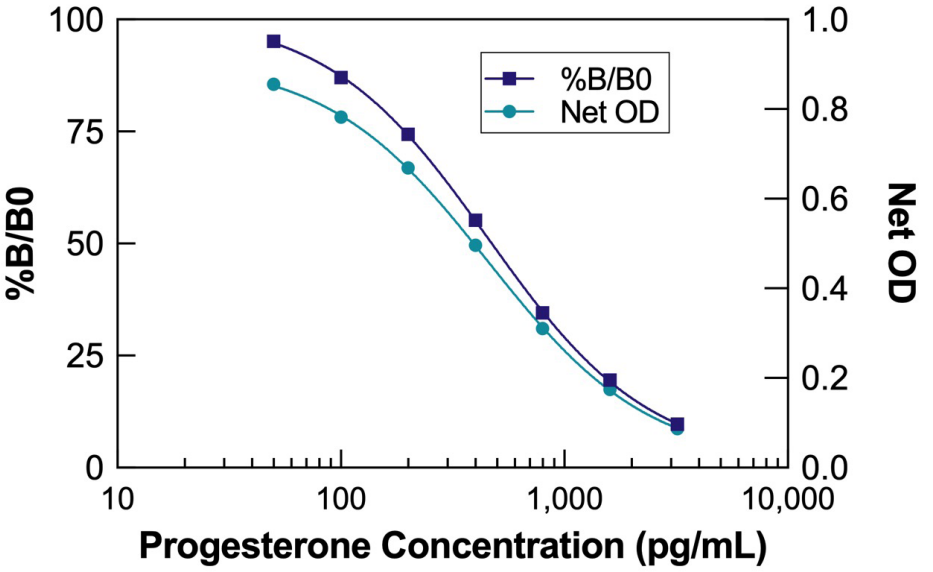
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Progesterone Conc. (pg/mL)
NSB	0.045	0	-	-
Standard 1	0.132	0.087	9.7	3,200
Standard 2	0.220	0.175	19.5	1,600
Standard 3	0.355	0.310	34.5	800
Standard 4	0.541	0.496	55.2	400
Standard 5	0.714	0.669	74.4	200
Standard 6	0.827	0.782	87.0	100
Standard 7	0.900	0.855	95.1	50
B0	0.944	0.899	100.0	0
Sample 1	0.303	0.258	28.6	1,017.1
Sample 2	0.552	0.507	56.4	381.8

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

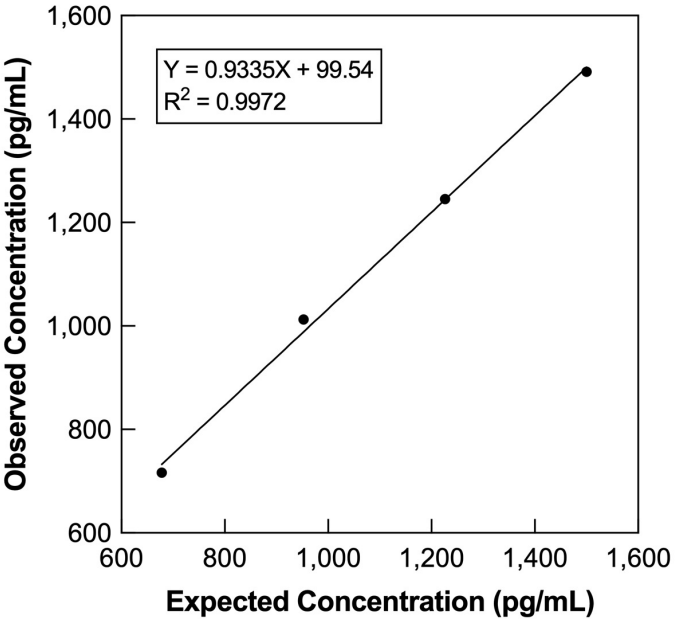
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 47.9 pg/mL.**

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 52.9 pg/mL.**

Linearity

Linearity was determined by taking two urine samples diluted with 1X Assay Buffer, one with a low diluted progesterone level of 404.1 pg/mL and one with a higher diluted level of 1,774.5 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%	678.2	716.3	105.6
60%	40%	952.3	1,012.5	106.3
40%	60%	1,226.4	1,245.2	101.5
20%	80%	1,500.5	1,491.6	99.4
			Mean Recovery	**103.2%**



Intra Assay Precision

Three human samples were diluted with 1X 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	1,038.0	3.5
2	405.0	3.1
3	260.6	5.1

Inter Assay Precision

Three human samples were diluted with 1X Assay Buffer and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated Progesterone concentrations were:

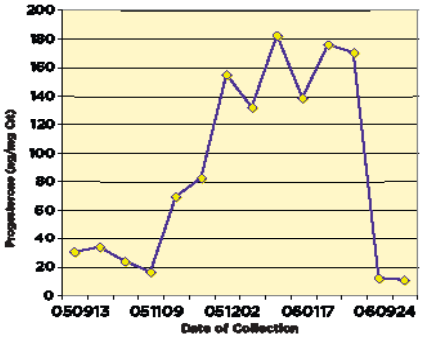
Sample	Progesterone Conc. (pg/mL)	%CV
1	1,057.0	4.1
2	406.0	5.9
3	261.9	7.0

SAMPLE VALUES

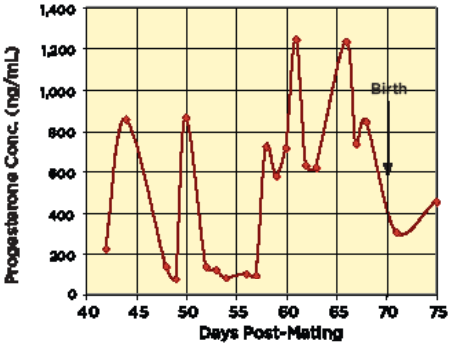
Eight human urine samples were tested in the assay, three came from pregnant women who were 10 weeks to 7 months pregnant. Adjusted neat concentrations of progesterone ranged from 4.041 to 164.1 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection Kit, K002-H1, the values ranged from 3.57 to 2,240 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%
3β-hydroxy-progesterone	172%
3α-hydroxy-progesterone	188%
11β-hydroxy-progesterone	2.7%
11α-hydroxy-progesterone	147%
5α-dihydroprogesterone	7.0%
Pregnenolone	5.9%
Corticosterone	< 0.1%
Androstenedione	< 0.1%

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 EIA kits for wildlife conservation research.