



ARBOR
ASSAYS

DetectX[®]

13,14-dihydro-15-keto-PGF_{2α}

(PGFM)

Enzyme Immunoassay Kit

1 Plate Kit
5 Plate Kit

Catalog Number K022-H1
Catalog Number K022-H5

SPECIES INDEPENDENT

Sample Types Validated:

**Fecal Extracts, Urine, Serum, Plasma and
Tissue Culture Media**

**Patent Pending - Leibniz Institute for Zoo &
Wildlife Research, Vienna**

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

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

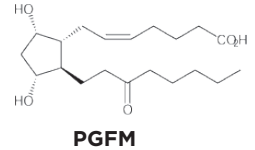
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BACKGROUND

In many species, uterine and placental Prostaglandin $F_{2\alpha}$ (PGF_{2 α}) is involved in the regulation of reproductive and pregnancy-related processes such as embryonic development, initiation of parturition, and resumption of ovarian activity¹. In domestic ruminants, uterine tissue is a primary source of PGF_{2 α} and secretion of uterine PGF_{2 α} is a key regulator for the cyclical regression of the corpus luteum²⁻⁴. Prostaglandin $F_{2\alpha}$ is metabolized to PGFM (13,14-dihydro-15-keto-PGF_{2 α}) during the first passage through the lungs⁵. PGFM has a longer half-life in peripheral circulation than PGF_{2 α} and has been applied as a useful analytical marker of PGF_{2 α} ⁶.



PGFM has been suggested as a useful non-invasive marker of pregnancy when measured in both urine and fecal samples⁷. It has been shown to be a precise, practical method for this application in these matrices. Parallel courses were obtained when comparing urinary and fecal PGFM in a variety of felids and other species, and only a simple dilution of fecal extracts is necessary prior to analyses. Fecal PGFM analyses may allow pregnancy diagnosis in captive and free-ranging felids. Recent evidence has suggested that PGFM may also be a useful pregnancy marker in other non-felid species⁸.

1. Frölich, JC., Wilson, TW., Sweetman, BJ., Smigel, M, Nies., AS, Carr, K., Watson, JT., and Oates, JA. (1975). "Urinary Prostaglandins. Identification and Origin." *J.Clin. Invest.*, 55:763-70.
2. McCracken, JA., Custer, EE., and Lamsa, JC. (1999) "Luteolysis: a neuroendocrine-mediated event." *Physiol Rev* . 79:263-323.
3. Schrick, FN., Inskeep. EK., and Butcher. RL. (1993) "Pregnancy rates for embryos transferred from early postpartum beef cows into recipients with normal estrous cycles." *Biol Reprod* 49:617-21.
4. Velez, JS., and Randel, RD. (1993) "Relationships between plasma progesterone and 13-14 dihydro-15-keto-prostaglandin- $F_{2\alpha}$ and resumption of ovarian activity during the postpartum period in Brahman cows." *Theriogenology* 39:1377-89.
5. Piper, PJ., Vane, JR., and Wyllie, JH. (1970) "Inactivation of prostaglandins by the lungs." *Nature* 225:600-4.
6. Ginther, OJ., Silva, LA., Araujo, RR., and Beg, MA. (2007). "Temporal associations among pulses of 13,14-di-hydro-15-keto-PGF_{2 α} , luteal blood flow, and luteolysis in cattle." *Biol Reprod.* 76:506-13.
7. Finkenwirth, C., Jewgenow, K., Meyer, HHD., Vargas, A., and Dehnhard, M. (2010) "PGFM (13,14-dihydro-15-keto-PGF_{2 α}) in pregnant and pseudo-pregnant Iberian lynx: A new noninvasive pregnancy marker for felid species." *Theriogenology* 73(4):530-540.
8. Dehnhard, M., Crossier, A., Kersey, D., Schwarzenberger, F., Walker, S., and Jewgenow, K. (2010) "Non-invasive pregnancy diagnosis in carnivores based on fecal prostaglandin $F_{2\alpha}$ metabolites." From: Proceedings of the First Annual Conference of the International Society of Wildlife Endocrinologists. Cincinnati, OH, November 4-6, 2010.

ASSAY PRINCIPLE

The DetectX[®] 13,14-dihydro-15-keto-PGF_{2α} (PGFM) Immunoassay kit is a patent pending assay from Dr. Martin Dehnhard at Leibniz Institute for Zoo & Wildlife Research, Vienna, Austria licensed exclusively and designed to quantitatively measure PGFM present in fecal extracts, urine, serum and plasma samples. Please read the complete kit insert before performing this assay. A PGFM 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 IgG. A PGFM-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a rabbit polyclonal antibody highly specific to PGFM to each well. After an hour incubation, the plate is washed and substrate is added. The substrate reacts with the bound PGFM-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 PGFM 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 Plate)	Catalog Number K002-H1
Urinary Creatinine Detection Kit (10 Plate)	Catalog Number K002-H5
Cortisol Enzyme Immunoassay Kits (Strip Wells)	Catalog Number K003-H1/H5
Cortisol Enzyme Immunoassay Kits (Whole Plate)	Catalog Number K003-H1W/H5W
Corticosterone Enzyme Immunoassay Kits	Catalog Number K014-H1/H5
Cortisone Enzyme Immunoassay Kits	Catalog Number K017-C1/H5



SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) with break-apart strips, coated with goat anti-rabbit IgG.

Kit K022-H1 **OR** -H5 1 **OR** 5 Each Catalog Number X016-1EA

PGFM Standard

13,14-dihydro-15-keto-PGF_{2α} at 32,000 pg/mL in a special stabilizing solution.

Kit K022-H1 **OR** -H5 125 **OR** 625 μL Catalog Number C085-125UL **OR** -625UL

DetectX® PGFM Antibody

A rabbit polyclonal antibody specific for PGFM.

Kit K022-H1 **OR** -H5 3 mL **OR** 13 mL Catalog Number C083-3ML **OR** -13ML

DetectX® PGFM Conjugate

A PGFM-peroxidase conjugate in a special stabilizing solution.

Kit K022-H1 **OR** -H5 3 mL **OR** 13 mL Catalog Number C084-3ML **OR** -13ML

Assay Buffer (or Concentrate)

One plate kit uses a ready-to-use Assay Buffer. Five plate kit uses a 5X concentrate that should be diluted with deionized or distilled water.

Kit K022-H1 28 mL Catalog Number X066-28ML
Kit K022-H5 28 mL (Conc) Catalog Number X067-28ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.

Kit K022-H1 **OR** -H5 30 mL **OR** 125 mL Catalog Number X007-30ML **OR** -125ML

TMB Substrate

Kit K022-H1 **OR** -H5 11 mL **OR** 55 mL Catalog Number X019-11ML **OR** -55ML

Stop Solution

A 1M solution of hydrochloric acid. CAUSTIC.

Kit K022-H1 **OR** -H5 5 mL **OR** 25 mL Catalog Number X020-5ML **OR** -25ML

Plate Sealer

Kit K022-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.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm, preferably with correction at between 570 and 590 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 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 extracts, urine, serum, and plasma samples.

Samples containing visible particulate should be centrifuged prior to using. Severely hemolyzed samples should not be used in this kit. All samples containing lipids may interfere with the measurement of PGFM. Samples containing high lipid content may be extracted as described below. A useful online resource for the extraction of bioactive lipids can be found at: http://lipidlibrary.aocs.org/topics/spe_alm/index.htm#ext.

PGFM is identical across all species and we expect this kit may measure PGFM from sources other than those tested. The end user should evaluate recoveries of PGFM in other samples being tested.

SAMPLE PREPARATION

Extracted 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 <5%.

Urine Samples

Urine samples should be diluted $\geq 1:8$ with the supplied Assay Buffer prior running in the assay.

Serum and Plasma Samples

Serum and plasma samples should be diluted $\geq 1:8$ with the supplied Assay Buffer prior running in the assay.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine PGFM concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer (for K022-H5)

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.

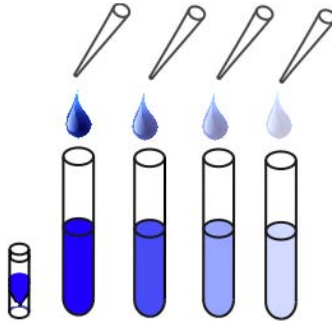
Wash Buffer

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 200 μL into tubes #2 to #7. **The PGFM stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 μL of the PGFM stock solution to tube #1 and vortex completely. Take 200 μL of the PGFM 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 PGFM 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
Assay Buffer (μL)	450	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (μL)	50	200	200	200	200	200	200
Final Conc (pg/mL)	3,200	1,600	800	400	200	100	50

ASSAY PROTOCOL

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 μ L of samples or standards into wells in the plate.
3. Pipet 75 μ L of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 μ L of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 μ L of the DetectX® PGFM Conjugate to each well using a repeater or multichannel pipet.
6. Add 25 μ L of the DetectX® PGFM Antibody to each well, **except the NSB wells**, using a repeater or multichannel 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 50% lower.
8. Aspirate the plate and wash 4 times with the wash buffer. Tap the plate dry on absorbent towels.
9. Add 100 μ L of the TMB Substrate to each well, using a repeater or a multichannel pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 μ L of the Stop Solution to each well, using a repeater or a multichannel pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm with correction between 570 and 590 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate PGFM 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.

TYPICAL DATA

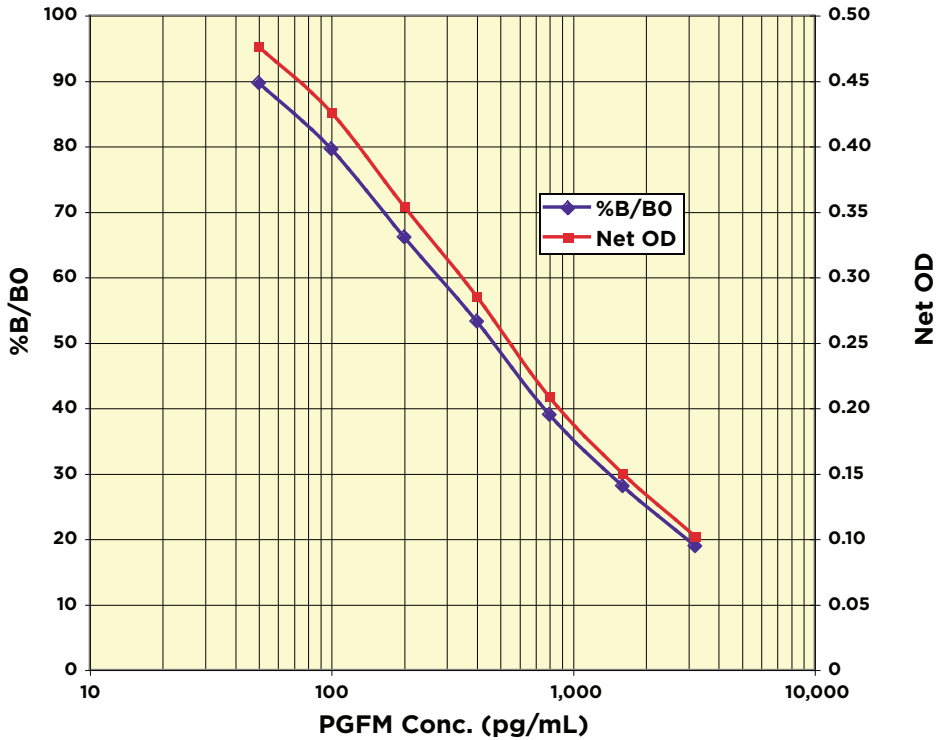
Sample	Mean OD	Net OD	% B/B0	PGFM Conc. (pg/mL)
NSB	0.044	0	-	-
Standard 1	0.148	0.104	19.8	3,200
Standard 2	0.208	0.164	31.3	1,600
Standard 3	0.248	0.204	38.9	800
Standard 4	0.331	0.287	54.8	400
Standard 5	0.395	0.351	67.0	200
Standard 6	0.482	0.438	83.6	100
Standard 6	0.526	0.482	92.0	50
B0	0.568	0.524	100.0	0
Sample 1	0.202	0.158	30.2	1,493
Sample 2	0.315	0.271	51.6	450.1

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

Conversion Factor: 100 pg/mL of PGFM is equivalent to 282.1 pM.



Typical Standard Curve



**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 BO and standard #7. The detection limit was determined at two (2) standard deviations from the BO along the standard curve.

Sensitivity was determined as 20.8 pg/mL.

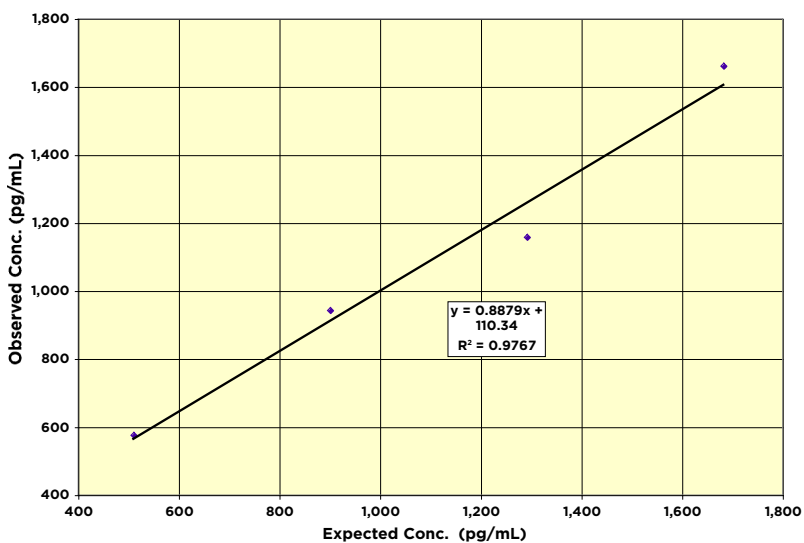
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration felid sample.

Limit of Detection was determined as 46.2 pg/mL

Linearity

Linearity was determined by taking two felid fecal samples, one with a low PGFM level of 119.8 pg/mL and one with a higher level of 2,074 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 Conc.	High Conc.	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	576.0	510.6	112.8
60%	40%	942.4	901.9	104.5
40%	60%	1,157.5	1,296.3	89.6
20%	80%	1,661.1	1,683.1	98.7
			Mean Recovery	101.4%



Intra Assay Precision

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

Sample	PGFM Conc. (pg/mL)	%CV
1	1,428.9	6.9
2	464.6	7.5
3	217.7	13.2

Inter Assay Precision - In Process

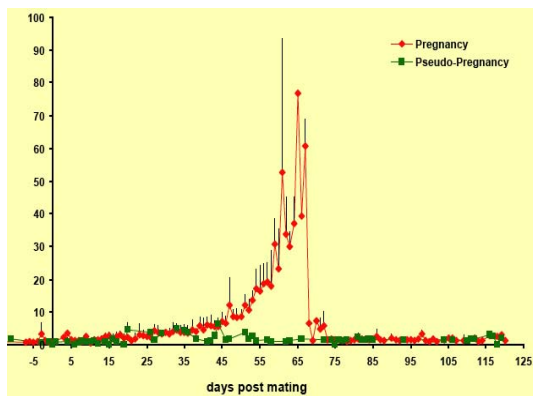
Three samples were diluted with Assay Buffer and run in duplicates in fourteen assays run over multiple days by three operators. The mean and precision of the calculated PGFM concentrations were:

Sample	PGFM Conc. (pg/mL)	%CV
1	1,485.2	6.8
2	472.2	9.6
3	189.7	12.6

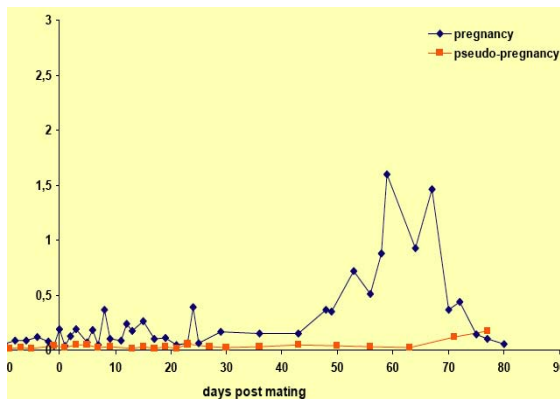
SAMPLE VALUES

PGFM has been analyzed in Iberian lynx urine and feces by Prof. Martin Dehnhart of the Leibniz Institute for Zoo & Wildlife Research, Vienna. The data below represent the PGFM concentrations found in either pregnant or pseudo-pregnant females.

Urinary PGFM (ng/mL)



Fecal PGFM ($\mu\text{g/g}$ feces)



CROSS REACTIVITY

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

Eicosanoid	Cross Reactivity (%)
13,14-dihydro-15-keto-Prostaglandin $F_{2\alpha}$ (PGFM)	100%
PGEM	1.5%
Prostaglandin $F_{2\alpha}$	0%
Prostaglandin E_2	0%
Tetranor-PGFM	0%
Tetranor-PGEM	0%
11 β -PGF $_{2\alpha}$	0%
PGF $_{2\beta}$	0%
PGAM	0%

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:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

E Mail Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

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