KAMIYA BIOMEDICAL COMPANY

PGFM Enzyme Immunoassay kit

For the quantitative determination of PGFM in fecal extracts, urine, serum, plasma and tissue culture media

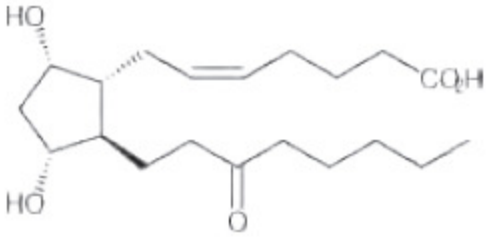
Cat. No. KT-741

For Research Use Only.

PRODUCT INFORMATION
PGFM Enzyme Immunoassay kit
Cat. No. KT-741

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

PGFM has been suggested as a useful non-invasive marker of pregnancy when measured in both urine and fecal samples. 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 some other non-felid species.



PGFM

PRINCIPLE
The 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) Immunoassay kit is 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 calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators 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 calibrators 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.

COMPONENTS
Coated Clear 96 Well Plates
A clear plastic microtiter plate(s) with break-apart strips, coated with goat anti-rabbit IgG.
1 Each

PGFM Calibrator
13,14-dihydro-15-keto-$PGF_{2\alpha}$ at 32,000 pg/mL in a special stabilizing solution.
125 µL

PGFM Antibody
A rabbit polyclonal antibody specific for PGFM.
3 mL

PGFM Conjugate

A PGFM-peroxidase conjugate in a special stabilizing solution.
3 mL

Assay Buffer
28 mL

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL

TMB Substrate
11 mL

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.
5 mL

Plate Sealer
1 Each

STORAGE

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 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 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.

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/spealm/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
The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Urine Samples
Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay.

Serum and Plasma Samples
Serum and plasma samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay.

Use all samples within 2 hours of preparation.

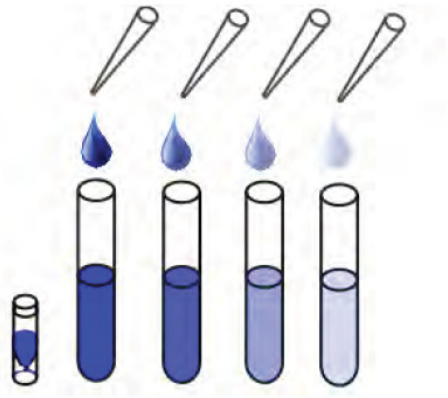
REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all calibrators 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.

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.

Calibrator 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 Calibrators 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 calibrator 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 calibrators 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 wells to act as maximum binding wells (B0 or 0 pg/mL).

5. Add 25 µL of the PGFM Conjugate to each well using a repeater pipet.

6. Add 25 µL of the PGFM 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 50% 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 PGFM concentration for each sample.

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration 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.

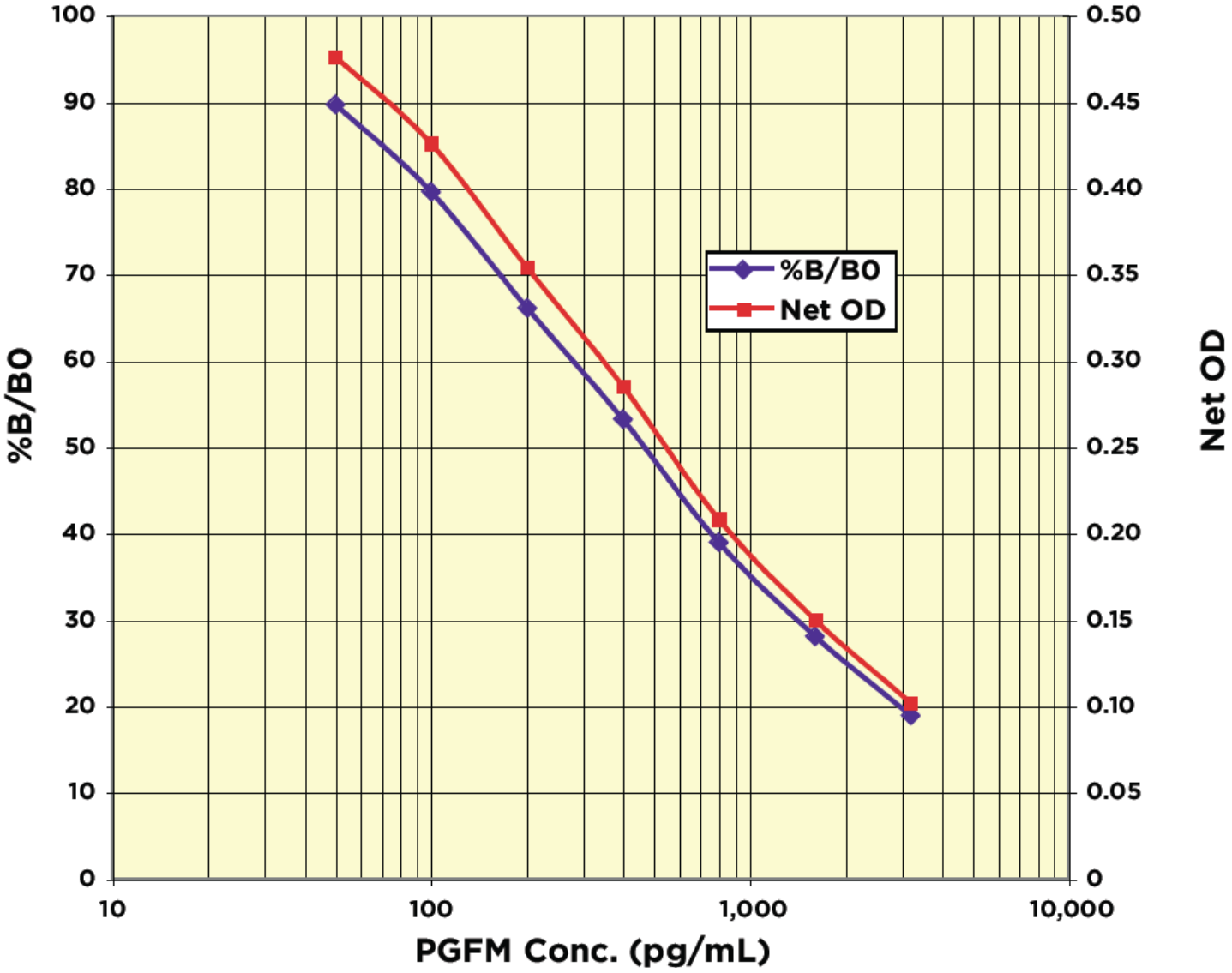
TYPICAL DATA

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 calibration curve for calculation of results. Do not use this data.

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

Typical Calibration Curve



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

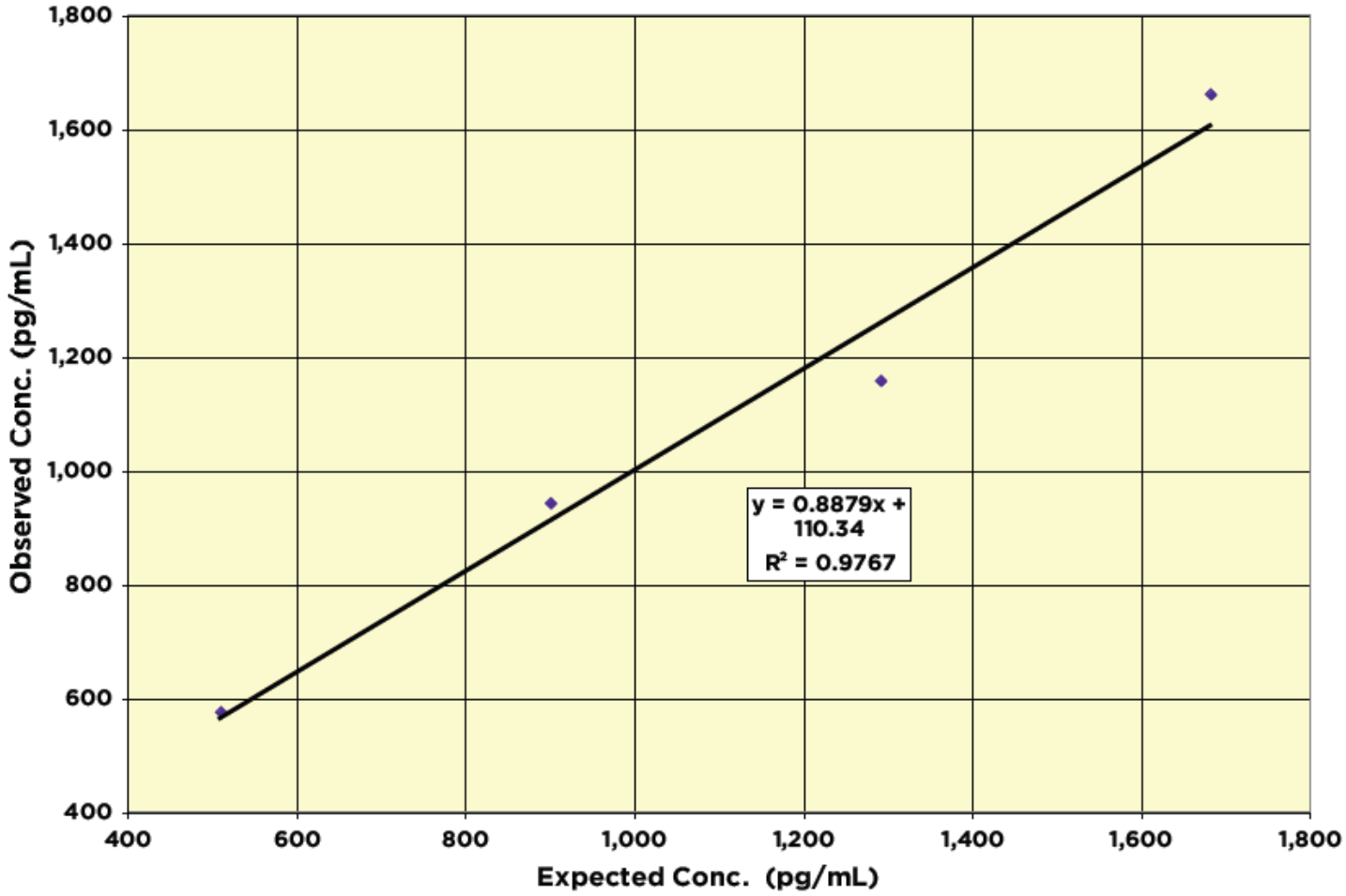
VALIDATION DATA

Sensitivity and Limit of Detection
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and calibrator #7. The detection limit was determined at two (2) standard deviations from the B0 along the calibration 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 calibrator 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

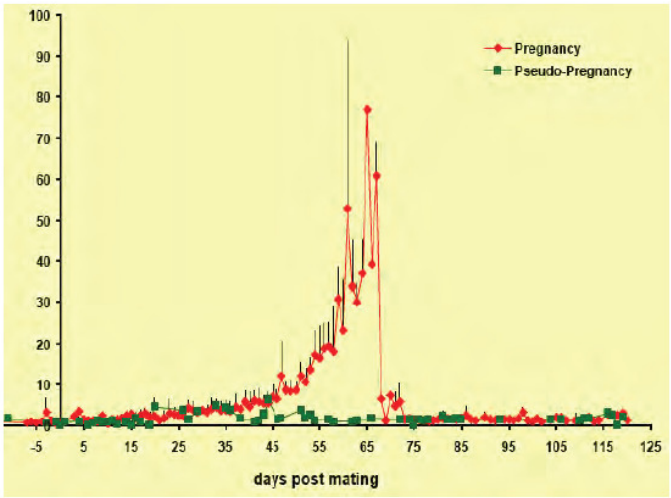
operators. The mean and precision of the calculated PGFM concentrations were:

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

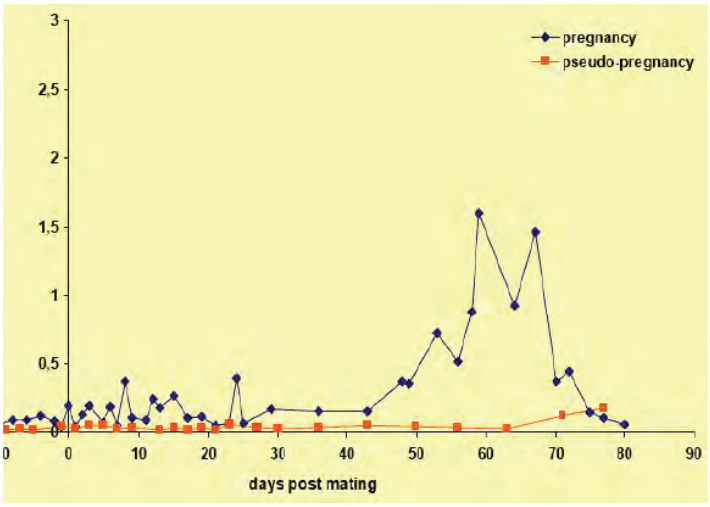
SAMPLE VALUES

PGFM has been analyzed in Iberian lynx urine and feces. The data below represent the PGFM concentrations found in either pregnant or pseudo-pregnant females.

Urinary PGFM (ng/mL)



Fecal PGFM (µg/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%
11ß-$PGF_{2\alpha}$	0%
$PGF_{2ß}$	0%
PGAM	0%

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com

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