13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) Enzyme Immunoassay Kit

User Manual

1 Plate Kit
Catalog # K3022-1

5 Plate Kit
Catalog # K3022-5

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INTENDED USE

The B-Bridge 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) Immunoassay kit (Cat # K3022-1 and K3022-5) is a patent pending assay from Dr. Martin Dehnhard at Leibniz Institute for Zoo & Wildlife Research, Berlin, Germany licensed exclusively and designed to quantitatively measure PGFM present in fecal extracts, urine, serum and plasma samples. This assay is species independent. Please read the complete kit insert before performing this assay.

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.

ASSAY PRINCIPLE

The B-Bridge 13,14-dihydro-15-keto-PGF2α (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.

1. Sample or standard added to well in microtiter plate coated with an antibody to capture rabbit IgG.
2. PGFM-peroxidase conjugate is added to each well containing either standards or sample
3. Add polyclonal antibody each well.
4. Incubate for 1 hour, wash plate, and add substrate to each well.
5. Substrate reacts with the bound PGFM-peroxidase conjugate and intensity of the generated color is measured at 450 nm wavelength.
6. Calculate PGE_2 concentration from standard curve.

KIT COMPONENTS

Component:	Cat #	K3022-1	K3022-5
Coated Clear 96 Well Plates		1 plate	5 plates
PGFM Standard (32,000 pg/mL)		125 uL	625 ul
PGFM Antibody		3 mL	13 mL
PGFM Conjugate		3 mL	13 mL
Assay Buffer (or Concentrate)		28 mL	28 mL
20X Wash Buffer Concentrate		30 mL	125 mL
TMB Substrate		11 mL	55 mL
Stop Solution (1M HCL): **Caution Caustic!**		5 mL	25 mL
Plate Sealer		1 each	5 each

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

MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Repeater pipet with disposable pipet tips capable of dispensing 25ul, 50ul, and 100ul
- 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 5.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

In all cases, please consult your institution's safety procedures for working with hazardous chemicals.

REAGENT PREPARATION

Allow the kit reagents to thaw and 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 Cat # K3022-5 only)
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

1. Label six test tubes as #1 through #7.

2. Pipet 450 µL of Assay Buffer into tube #1 and 200 µL of Assay Buffer into tubes #2 - #7.

3. Carefully add 50 µL of the PGFM stock solution to tube #1 and vortex completely.
   Note: The PGFM stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.

4. Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely.

5. 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, respectively.

Use all Standards within 2 hours of preparation.

Reagent	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Assay Buffer	**450 µl**	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
PGFM Stock	50 µl						
Standard 1		200 µl					
Standard 2			200 µl				
Standard 3				200 µl			
Standard 4					200 µl		
Standard 5						200 µl	
Standard 6							200 µl
Final PGFM Concentration (pg/mL)	3,200	1,600	800	400	200	100	50

SAMPLE PREPARATION

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.

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

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

Extracted Solid Samples
Weigh out ≥ 0.2 gm of dried fecal solid into a tube.
Add 1 mL of Ethanol (or Ethyl Acetate) for every 0.1 gm of solid
Vortex for at least 30 minutes.
Centrifuge samples at 5,000 rpm for 15 minutes, then transfer supernatant to a clean tube.
Evaporate supernatant*.
Store dried extracted samples at -20°C in a desiccator.
Just before assaying, dissolve extracted sample with 100 µL ethanol and add at least 400 µL Assay Buffer. Vortex well, then allow to rest 5 minutes at room temperature. Repeat 2 times to ensure complete resuspension. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%. Assay reconstituted diluted samples immediately.

Determine the extraction efficiency by comparing the concentration of the steroid measured in the extracted Control (Control Spike - Control Sample) with the concentration of steroid before extraction

*If only a portion of the organic solvent is being evaporated, ensure final amounts of measured steroid per gm solid accounts for volume of solution evaporated.

Use all samples within 2 hours of preparation.

ASSAY PROTOCOL

1. Pipet 50 µL of samples or standards into the appropriate number of wells in the plate.
2. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
3. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
4. Add 25 µL of the PGFM Conjugate to each well using a repeater or multichannel pipet.
5. Add 25 µL of the PGFM Antibody to each well, **except the NSB wells**, using a repeater or multichannel pipet.
6. 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.
7. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
9. Incubate the plate at room temperature for 30 minutes without shaking.
10. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

11. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

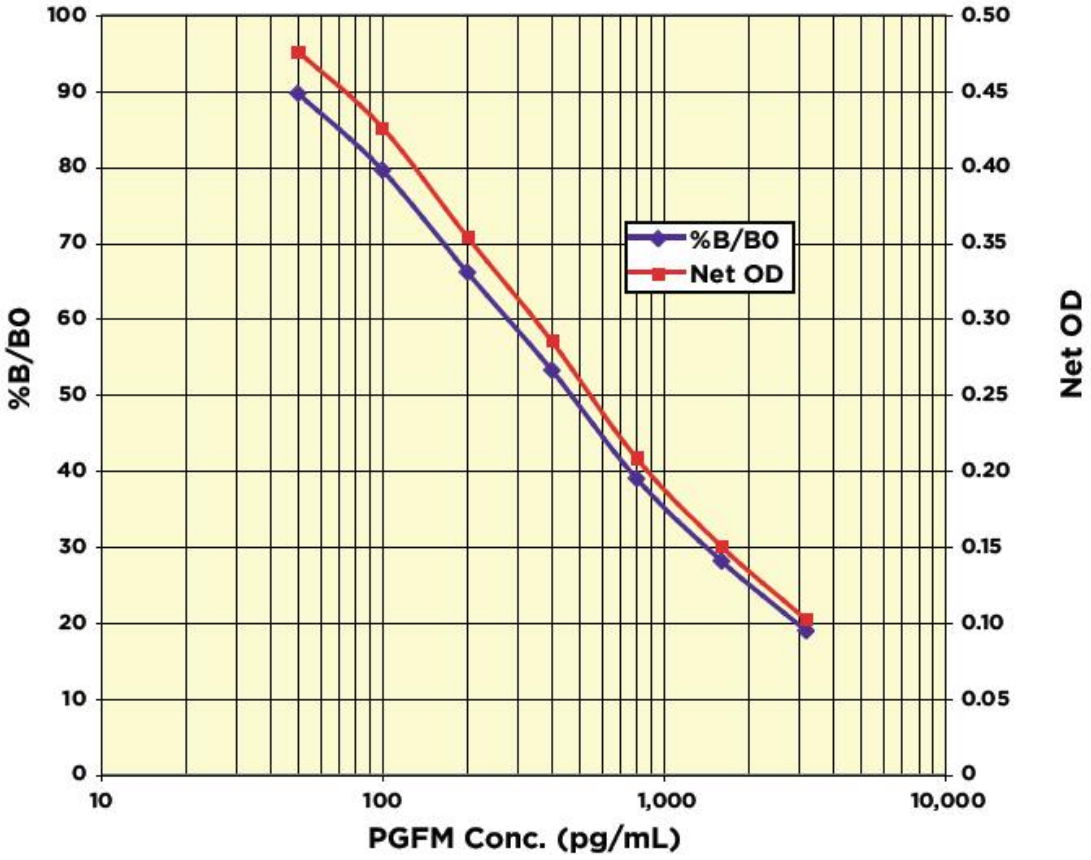
12. Use the plate reader's built-in 4PLC software capabilities to calculate PGFM concentrations for each sample.

CALCULATIONS

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.

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

TYPICAL STANDARD CURVE: EXAMPLE ONLY



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