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Pregnanediol-3-Glucuronide (PDG) ELISA Kit

07/20

(Catalog # E4851-100, 96 assays, Store at 4°C)

I. Introduction:

Pregnanediol Glucuronide, C27H44O8, also known as PDG (5β-Pregnan-3a, 20a-diol 3-glucosiduronate) is the major metabolite of progesterone. 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. BioVision's PDG ELISA kit is a competitive ELISA assay for the quantitative measurement of PDG in urine and fecal samples. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. A PDG-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 PDG to each well. After incubation the plate is washed and substrate is added. The substrate reacts with the bound PDG-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.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of PDG.
Detection Range: 0.391 - 50 ng/mL
Sensitivity: 0.180 ng/mL
Detection Limit: 0.320 ng/mL

III. Specificity:

Universal

IV. Sample Type:

Plasma, Urine and Tissue Culture Media

V. Kit Contents:

Components	E4851-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4851-100-1
Standard	125 µl	E4851-100-2
PDG Antibody	3 ml	E4851-100-3
PDG Conjugate	3 ml	E4851-100-4
Assay Buffer (5X)	28 ml	E4851-100-5
Wash Buffer (20X)	30 ml	E4851-100-6
TMB Substrate	11 ml	E4851-100-7
Stop Solution	5 ml	E4851-100-8
Plate Sealer	1	E4851-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 months. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **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.
2. **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 for 3 months at room temperature.
3. **Standard Preparation:**

- Add 50 µl of the PDG stock solution to 450 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 7 vials of standards (tube #2-8) by adding 200 µl of Assay Buffer. Take 200 µl of the PDG solution from above vial #1 and add to vial #2. Vortex thoroughly. Perform serial dilutions for vial #3 to #8 to make the standard curve within the range of this assay.
- Suggested standard points are: 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.391 ng/mL.
- Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**450**	200	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 5	Std 6
Vol of Addition (µL)	**50**	200	200	200	200	200	200	200
Final Conc (ng/mL)	50	25	12.5	6.25	3.125	1.563	0.781	0.391

4. Sample Preparation:

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Extracted serum and plasma**: Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio. Mix solutions by vortexing for 2 minutes. Allow ether layer to separate for 5 minutes. Freeze samples in a dry ice/ethanol bath and pipet off the ether solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions. Dry pooled ether samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C. Redissolve samples at room temperature in the Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.
- **Urine**: Urine samples should be diluted at least 1:5 with the provided Assay Buffer.
- **Tissue Culture Media:** For measuring PDG 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 depending on PDG levels.
- **Dried Fecal Samples:** Ensure that the sample is completely dry and powder the sample to improve extraction recovery. Remove any large particles, such as grass if possible. We suggest checking the efficiency of extraction by preparing a steroid solution of known concentration in the kit Assay Buffer (AB). Please refer to Extraction Efficiency section below for details. Extract samples and Extraction Efficiency Controls with ethanol or ethyl acetate as follows:
  1. Weigh out ≥ 0.2 gm of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
  2. Add 1 mL of ethanol (or ethyl acetate) for every 0.1 gm of solid (0.1 gm fecal solid/mL) and seal.
  3. Shake vigorously for at least 30 minutes.
  4. Centrifuge samples at 5,000 rpm for 15 minutes at 4°C. Reserve supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed**.**
  5. These are general guidelines, please adjust volumes as required. Add 100 µL of extracted sample supernatant from step 4 into a minimum volume of 400 µL assay buffer (Total Reconstitution Vol. = 100µL+400µL). Vortex well and allow to rest 5 minutes at room temperature. Vortex and let rest for 5 minutes twice more to ensure complete steroid solubility.

  Note: The ethanol concentration in the final Assay Buffer dilution added to the well should be < 1%

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 50 µl of samples or standards into wells in the plate. Pipet 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the PDG Conjugate to each well. Add 25 µl of the PDG Antibody to each well, except the NSB wells.
4. 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.
5. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
6. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes without shaking.
7. Add 50 µl of the Stop Solution to each well.
8. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

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 non-specific binding well (NSB). The sample concentrations obtained, calculated from the %B/B_0 curve, and should be multiplied by the dilution factor to obtain neat sample values.

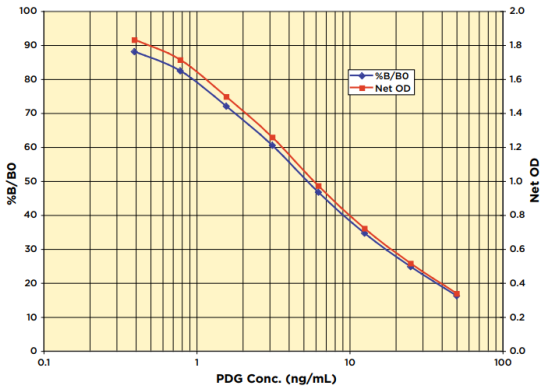

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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XI. VALIDATION DATA:

Recovery Rate: Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted pregnanediol-3-glucuronide (PDG) level of 1.70 ng/mL and one with a higher diluted level of 30.7 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	24.9	23.8	95.4
60%	40%	19.1	16.9	88.3
40%	60%	13.3	12.1	90.7
20%	80%	7.5	6.7	89.1
			Mean Recovery	**90.9%**

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

Sample	PDG Conc. (ng/mL)	%CV
1	12.5	2.9
2	4.0	3.7
3	1.5	5.7

Inter Assay Precision:
Three urine samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by four operators. The mean and precision of the calculated PDG concentrations were:

Sample	PDG Conc. (ng/mL)	%CV
1	12.3	6.4
2	3.9	5.2
3	1.3	7.5

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

Steroid	Cross Reactivity (%)
Pregnanediol-3-glucuronide	100%
20α-hydroxyprogesterone	44.8%
20β-hydroxyprogesterone	3.16%
Progesterone	0.2%
Testosterone	0.2%
Cortisol	0.07%
17β-Estradiol	0.07%
Estrone-3-glucuronide	0.07%

XII. RELATED PRODUCTS:

- Progesterone (human) ELISA Kit (K7414)
- Progesterone receptor (PGR) (Human) ELISA Kit (K4270)
- Estradiol (rat) ELISA Kit (K3831)
- Estrogen (Human) ELISA Kit (K4264)
- Androstenedione ELISA Kit (E4615)

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