KAMIYA BIOMEDICAL COMPANY

Estrone-3-Glucuronide (E1G) EIA Kit

For the quantitative determination of E1G and its metabolites in dried fecal extracts, urine, extracted serum/plasma and tissue culture media

Cat. No. KT-721

For Research Use Only.

PRODUCT INFORMATION
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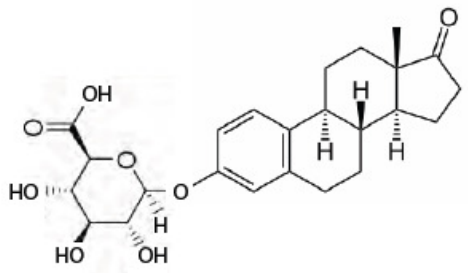
BACKGROUND
Estrone-3-glucuronide, $C_{24}H_{30}O_8$, (1,3,5(10)-estratrien-3-ol-17-one glucosiduronate, E1G) is the principle secreted form of circulating estradiol in mammals.

Ovulation is the critical event of each menstrual cycle that occurs during the reproductive life of healthy females and the ovum can only be fertilized during the short period of time in which it is viable. Spermatozoa also have a limited biological life-span and the ease with which they can ascend the female genital tract is largely dependent upon the quality of mucus secreted by the cervix, which is under hormonal control. The three phases of the menstrual cycle are: (i) an initial phase when there is only a low risk that would enable viable spermatazoa to survive and reach the ovum, (ii) a phase when the chance of fertilization is at a maximum, the fertile period, and (iii) a time of absolute infertility when the ovum is no longer viable. Clinical studies have indicated the utility of measuring estrone-3-glucuronide (E1G) and pregnanediol-3a-glucuronide (PDG) in samples of urine to monitor ovarian function in females.

Estrone-3-Glucuronide, E1G



There is substantial evidence supports an association of endogenous reproductive hormone exposure with increased risk of reproductive cancers. Greater estrogen exposure, assessed via indirect indicators such as number of years spent having menstrual cycles or direct indicators such as hormone measures, is associated with increased risk for cancers of the breast and ovary.

PRINCIPLE
The Estrone-3-Glucuronide (E1G) Immunoassay kit uses a specifically generated antibody to measure E1G and its metabolites in urine and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum, plasma, or saliva samples without extraction. The kit will quantitatively measure E1G present in diluted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An E1G 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 antibodies. An E1G-peroxidase conjugate is added to the calibrators and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to E1G to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound E1G-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 450nm wavelength. The concentration of the E1G 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
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.
1 Each

Estrone-3-Glucuronide (E1G) Calibrator
Estrone-3-Glucuronide (E1G) at 10,000 pg/mL in a special stabilizing solution.
125 µL

Estrone-3-Glucuronide (E1G) Antibody
A rabbit polyclonal antibody specific for Estrone-3-Glucuronide.
3 mL

Estrone-3-Glucuronide (E1G) Conjugate
An Estrone-3-Glucuronide-peroxidase conjugate in a special stabilizing solution.
3 mL

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
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, urine and tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Estrone-3-glucuronide can be assayed in solid sample types.

Estrone-3-glucuronide (E1G) is identical across all species and we expect this kit to measure estrone-1-glucuronide from all sources. The end user should evaluate recoveries of E1G in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

We would recommend the following protocol for serum and plasma.

1. Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio.
2. Mix solutions by vortexing for 2 minutes. Allow ether layer to separate for 5 minutes.
3. 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.
4. 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.
5. 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.

Dried Fecal Samples

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

Urine Samples

Urine samples should be diluted at least 1:8 times with the provided Assay Buffer.

Tissue Culture Media

For measuring estrone-1-glucuronide in tissue culture media (TCM), samples should be read off a calibration curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine estrone-3-glucuronide concentrations. 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 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

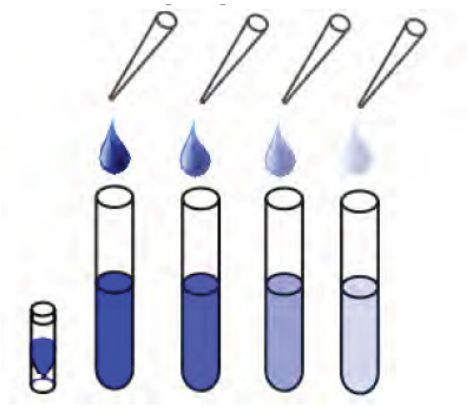
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

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 Estrone-3-Glucuronide stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the estrone-3-glucuronide stock solution to tube #1 and vortex completely. Take 200 µL of the estrone-3-glucuronide 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 estrone-3-glucuronide in tubes 1 through 7 will be 1,000, 500, 250, 125, 62.5, 31.25 and 15.625 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)	1,000	500	250	125	62.5	31.25	15.625

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 (Bo or 0 pg/mL).

5. Add 25 µL of the Estrone-3-Glucuronide Conjugate to each well using a repeater pipet.

6. Add 25 µL of the Estrone-3-Glucuronide 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 2 hours. If the plate is not shaken signals bound will be approximately 35% 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 estrone-1- glucuronide 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.

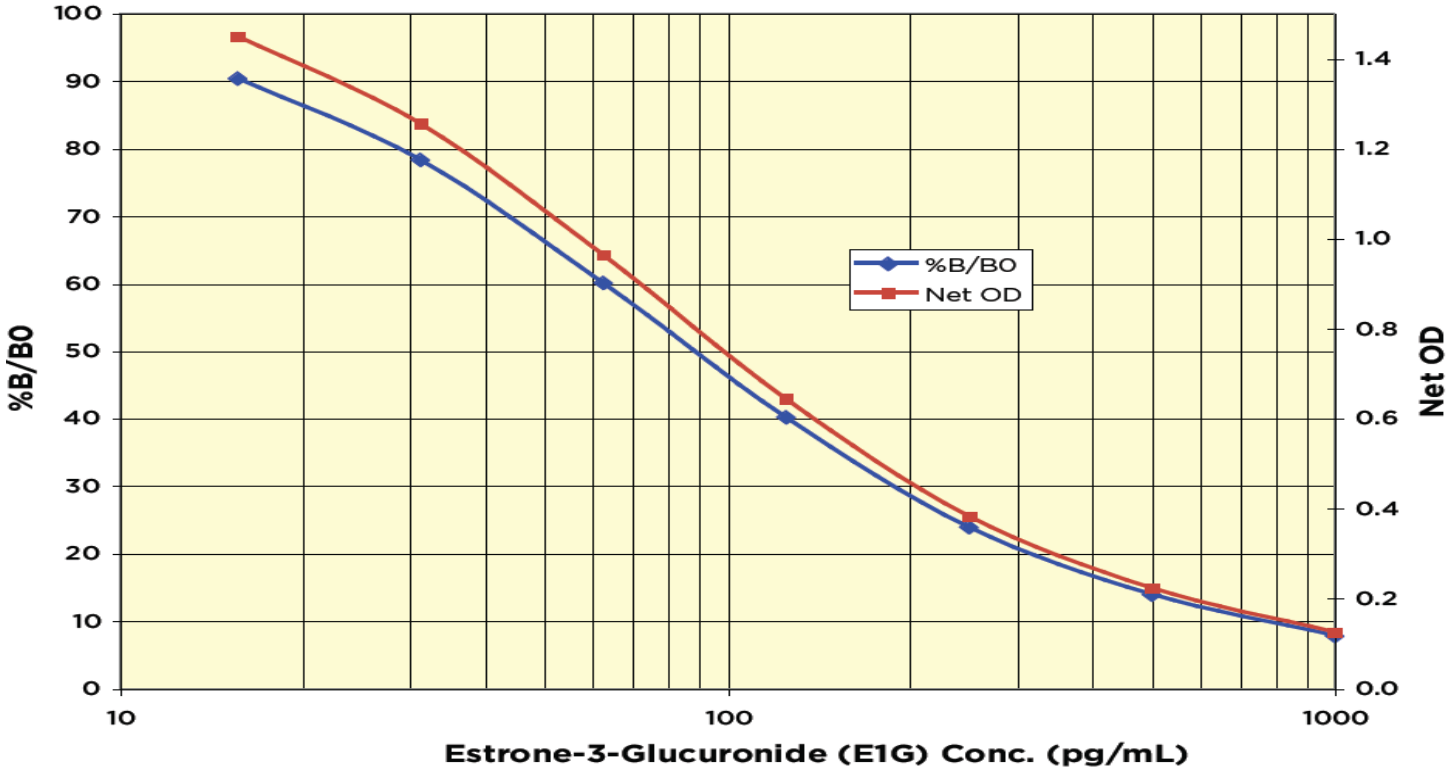
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	E1G Conc. (pg/mL)
NSB	0.042	0	-	-
Standard 1	0.167	0.125	7.8	1,000
Standard 2	0.265	0.223	13.9	500
Standard 3	0.425	0.383	23.9	250
Standard 4	0.686	0.644	40.2	125
Standard 5	1.006	0.964	60.1	62.5
Standard 6	1.298	1.256	78.4	31.25
Standard 7	1.491	1.449	90.4	15.625
B0	1.645	1.603	100.0	0
Sample 1	0.416	0.374	23.3	258.5
Sample 2	1.184	1.142	71.2	41.8

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

Conversion Factor: 100 pg/mL of E1G is equivalent to 224 pM.

Typical Calibration Curves



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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 7.38 pg/mL.

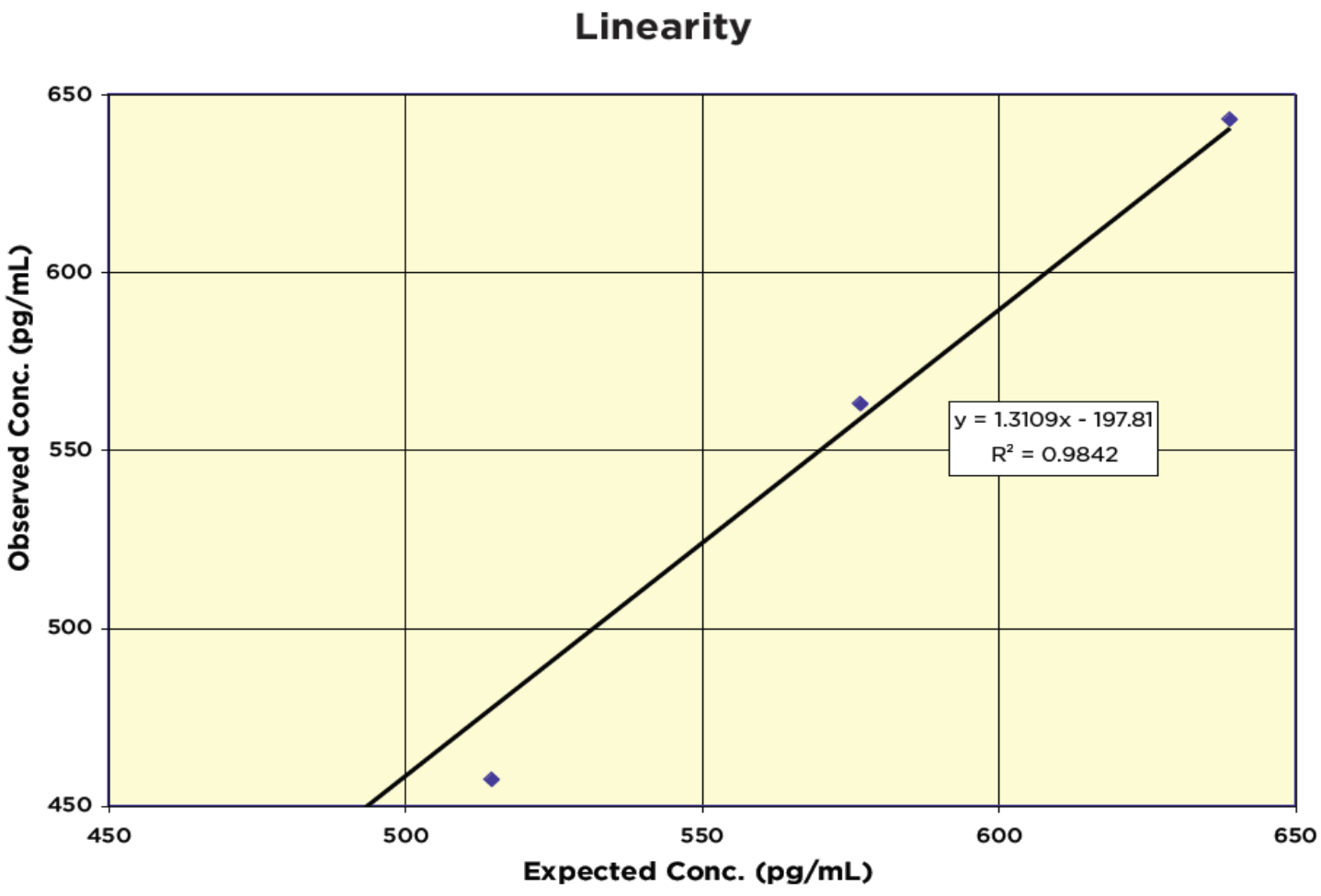
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero calibrator and a low concentration human urine sample.

Limit of Detection was determined as 8.76 pg/mL

Linearity

Linearity was determined by taking two urine samples diluted 1:20 with Assay Buffer, one with a low diluted estrone-3-glucuronide (E1G) level of 390.2 pg/mL and one with a higher diluted level of 701.1 pg/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	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	643.0	638.9	100.6
60%	40%	563.0	576.7	97.6
40%	60%	457.4	514.5	88.9
20%	80%	406.5	452.4	89.9
			Mean Recovery	**94.3%**



Intra Assay Precision

Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Estrone-3-glucuronide (E1G) concentrations were:

Sample	E1G Conc. (pg/mL)	%CV
1	241.1	3.1
2	70.7	3.5
3	39.9	4.7

Inter Assay Precision

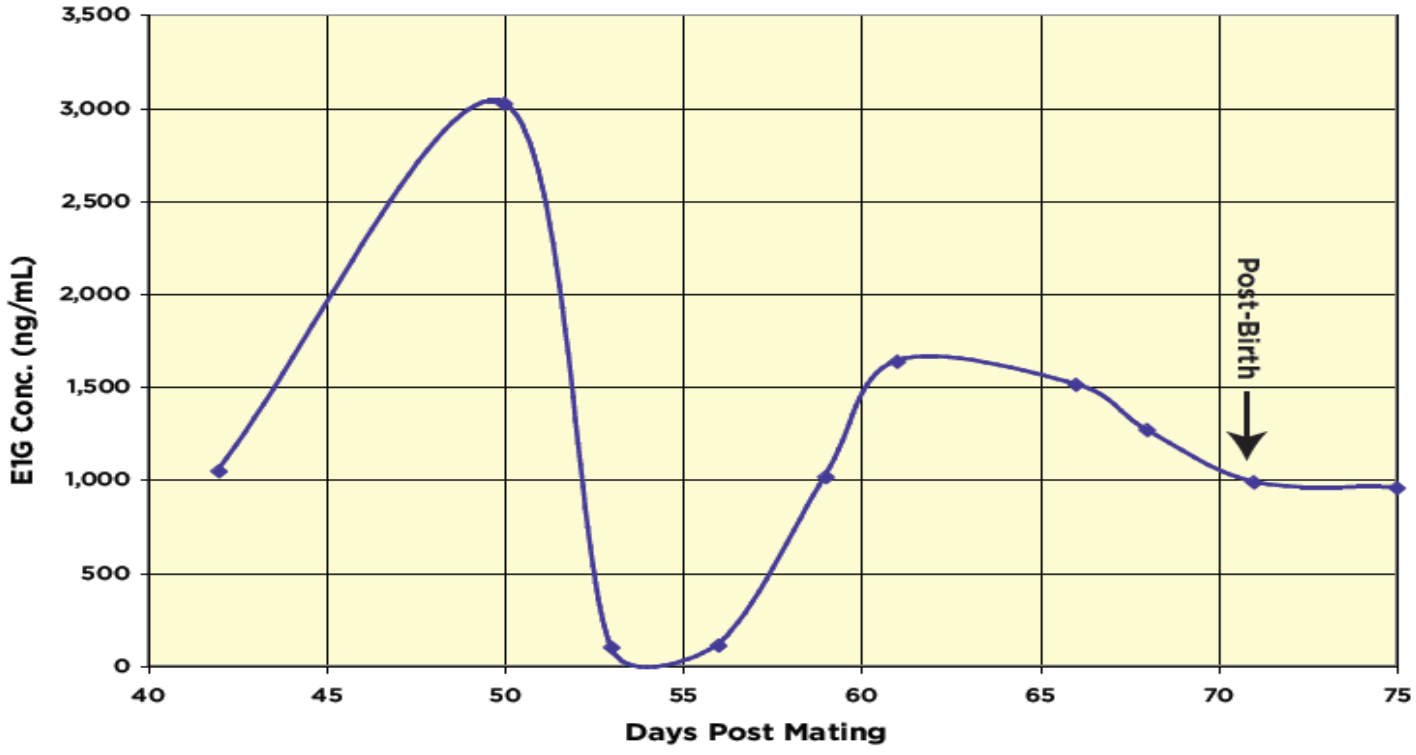
Three urine samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by four operators. The mean and precision of the calculated Estrone-3-glucuronide (E1G) concentrations were:

Sample	E1G Conc. (pg/mL)	%CV
1	252.8	4.7
2	70.7	5.9
3	38.9	6.3

SAMPLE VALUES

Ten urine samples from various species were tested in the assay. Adjusted neat concentrations of Estrone-3-Glucuronide (E1G) ranged from 0.831 to 19.3 ng/mL.

Fecal samples from Camarina, a female Iberian Lynx, were extracted and tested in the assay.



CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
Estrone-3-glucuronide (E1G)	100%	Estradiol-17-Sulfate	0.1%
Estrone-3-Sulfate (E1S)	66.6%	Progesterone	< 0.1%
Estrone	238%	Estriol	< 0.1%
17ß-Estradiol	7.8%	Cortisol	< 0.1%
Estradiol-3-Glucuronide	3.8%	Testosterone	< 0.1%
Estradiol-3-Sulfate	3.3%	Pregnanediol	< 0.1%

FOR RESEARCH USE ONLY

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