ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Estrone-3-Glucuronide (E1G)
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K036-H1

5 Plate Kit Catalog Number K036-H5

Species Independent

Multi-Format Kit

Sample Types Validated:

**Dried Fecal Extracts, Urine,
Extracted Serum/Plasma, and
Tissue Culture Media**

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

K036-H WEB 191002

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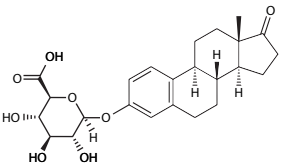
BACKGROUND

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[1-4]. 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[5-7]. Greater estrogen exposure, assessed via indirect indicators such as number of years spent having menstrual cycles[6] or direct indicators such as hormone measures[7], is associated with increased risk for cancers of the breast and ovary[8,9].

1. Adlercreutz, H., Lehtinen, T. and Kairento, A.-L. Prediction of ovulation by urinary estrogen assays. J Steroid Biochem 1980, 12:395-401.

2. Stanczyk, F.Z., Miyakawa, I. and Goebelsmann, U. Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. Am J Obstet Gynecol, 1980, 137:443-450.

3. Collins, W.P., Branch, C.M., Collins, P.O. and Sallam H.N. Biochemical indices of the fertile period in women. Int J Fertil., 1981, 26:196-202.

4. Brown, J.8. and Gronow, M. Endocrinology of ovulation prediction, in Clinical Reproductive Endocrinology (R.P. Shearman, Editor). Churchill Livingstone, Edinburgh, 1985, p. 165-184.

5. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. Cancer Res 1982; 42:3232–9.

6. Key TJ. Hormones and cancer in humans. Mutat Res 1995;333:59–67.

7. Ursin G, et al. Do urinary estrogen metabolites reflect the differences in breast cancer risk between Singapore Chinese and United States African-American and White women? Cancer Res 2001;61:3326–9.

8. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. Cancer Epidemiol Biomarkers Prev 2005;14:98–107.

9. Persson I. Estrogens in the causation of breast, endometrial and ovarian cancers—evidence and hypotheses from epidemiological findings. J Steroid Biochem Mol Biol 2000;74:357–64.

ASSAY PRINCIPLE

The DetectX® Estrone-3-Glucuronide (E1G) Enzyme 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 or plasma 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 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 antibodies. Two formats can be chosen. Using 50 µL of sample or standards provides a standard curve from 1,000 to 15.625 pg/mL. A 100 µL standard and sample volume format will give a standard curve from 250 to 3.906 pg/mL. An E1G-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 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 450 nm 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.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone ELISA Kits	K053-H1/H5
Aldosterone ELISA & Chemiluminescent ELISA Kits	K052-H1/H5, K052-C1/C5
Allopregnanolone ELISA Kits	K061-H1/H5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Dehydro-epiandrosterone sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Estradiol Non-Invasive & Serum ELISA Kits	K030-H1/H5, KB30-H1/H5
Estrone ELISA Kits	K031-H1/H5
Estrone-3-Sulfate (E1S) ELISA Kits	K038-H1/H5
Levonorgestrel ELISA Kits	K058-H1/H5
Oxytocin ELISA & Chemiluminescent ELISA Kits	K048-H1/H5, K048-C1/C5
PGFM (13,14-dihydro-15-keto-Prostaglandin F2alpha) ELISA Kits	K022-H1/H5
Pregnanediol Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progestrone ELISA Kits	K025-H1/H5
Progestrone Metabolites ELISA Kits	K068-H1/H5
Prolactin ELISA Kit	K040-H1
Testosterone ELISA Kits	K032-H1/H5
Urinary Creatinine Detection Kits (2 or 10 Plates)	K002-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.

Kit K036-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

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

Kit K036-H1 **or** -H5	125 µL **or** 625 µL	Catalog Number C124-125UL **or** -625UL

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

Kit K036-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C122-3ML **or** -13ML

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

Kit K036-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C123-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.

Kit K036-H1 **or** -H5	28 mL **or** 55 mL	Catalog Number X065-28ML **or** -55ML

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

Kit K036-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K036-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

Stop Solution
A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K036-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K036-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.

Repeater pipet with disposable tips capable of dispensing 25, 50 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 as prepared on page 8.

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 by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols.

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 have 3 detailed Extraction Protocols available on our website at: www.ArborAssays.com/resources/#protocols as a PDF file entitled "Steroid Serum/Plasma Extraction Protocol". 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

We have a detailed Extraction Protocol available on our website at: www.ArborAssays.com/resources/#protocols. 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. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection Kits, K002-H1/H5.

Tissue Culture Media

For measuring estrone-1-glucuronide 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. We have validated the assay using RPMI-1640.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

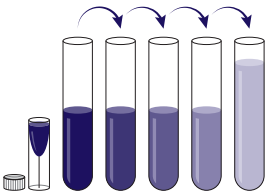
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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.

Standard Preparation - 50 µL Assay Format

Label 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 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)	1,000	500	250	125	62.5	31.25	15.625

Standard Preparation - 100 µL Assay Format

Label test tubes as #1 through #7. Pipet 585 µL of Assay Buffer into tube #1 and 300 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 15 µL of the estrone-3-glucuronide stock solution to tube #1 and vortex completely. Take 300 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 250, 125, 62.5, 31.25 , 15.625, 7.813 and 3.906 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)	585	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	15	300	300	300	300	300	300
Final Conc (pg/mL)	250	125	62.5	31.25	15.625	7.813	3.906

ASSAY PROTOCOL - 50 µL AND 100 µL FORMATS

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine estrone-3-glucuronide concentrations.

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 µL of samples or standards (100 µL for alternate format) into wells in the plate.
3. Pipet 75 µL of Assay Buffer (125 µL for alternate format) into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer (100 µL for alternate format) into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Estrone-3-Glucuronide Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® 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.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

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.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-estrone-3-glucuronide-(e1g)-eia-kit.assay

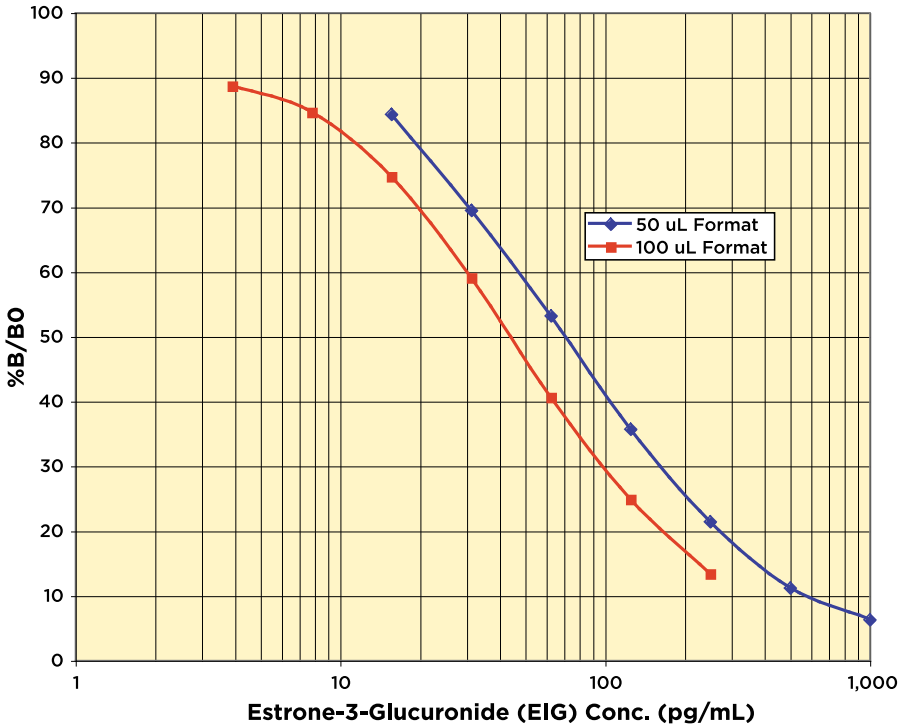
TYPICAL DATA

	50 µL Assay				100 µL Assay			
Sample	**Mean OD**	**Net OD**	**% B/B0**	**E1G Conc. (pg/mL)**	**Mean OD**	**Net OD**	**% B/B0**	**E1G Conc. (pg/mL)**
NSB	0.089	0	-	-	0.083	0	-	-
Standard 1	0.164	0.075	6.3	1,000	0.206	0.118	13.4	250
Standard 2	0.221	0.133	11.2	500	0.307	0.219	24.9	125
Standard 3	0.342	0.253	21.4	250	0.445	0.357	40.6	62.5
Standard 4	0.511	0.423	35.7	125	0.607	0.519	59.1	31.25
Standard 5	0.719	0.630	53.2	62.5	0.744	0.656	74.7	15.625
Standard 6	0.913	0.824	69.5	31.25	0.831	0.742	84.6	7.813
Standard 7	1.087	0.999	84.3	15.625	0.867	0.779	88.7	3.906
B0	1.274	1.185	100.0	0	0.966	0.878	100.0	0
Sample 1	0.344	0.255	21.5	248.6	0.213	0.130	14.7	231.2
Sample 2	0.867	0.778	65.7	37.6	0.581	0.498	56.2	34.8

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

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

Typical Standard Curves



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

VALIDATION DATA

Sensitivity and Limit of Detection for the 50 µL Format

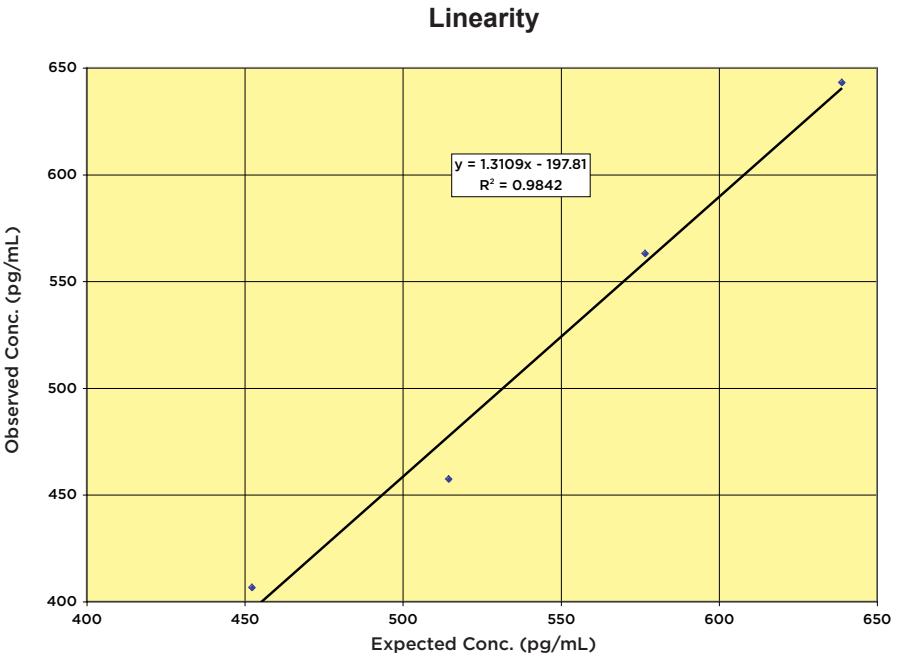
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 7.38 pg/mL.**

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 standard and a low concentration human urine sample. **Limit of Detection was determined as 8.76 pg/mL.**

Linearity for the 50 µL Format

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	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	638.9	643.0	100.6
60%	40%	576.7	563.0	97.6
40%	60%	514.5	457.4	88.9
20%	80%	452.4	406.5	89.9
			Mean Recovery	**94.3%**



Intra Assay Precision for the 50 µL Format

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 for the 50 µL Format

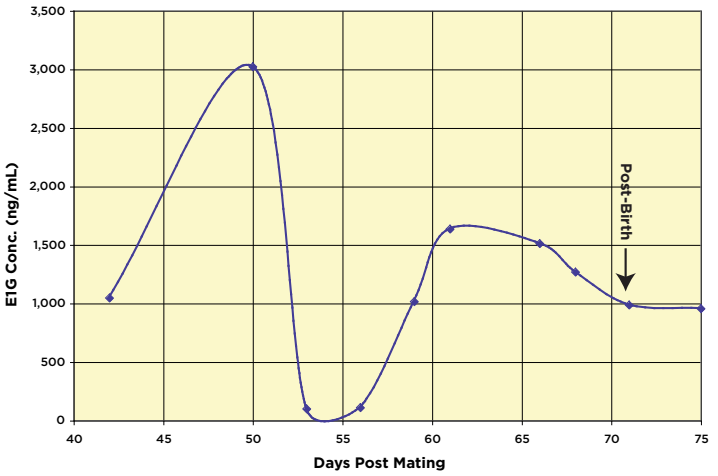
Three urine samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by four operators. The mean and precision of the calculated Estrone-3-glucuronide (E1G) concentrations were:

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. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection kit, K002-H1, the values ranged from 7.08 to 732.7 ng/mg creatinine.

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



The Iberian Lynx samples were the kind gift from Dr. Martin Dehnhard, Leibniz Institute for Zoo and Wildlife Research, Berlin.

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

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:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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