ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

17β-Estradiol
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K030-H1

5 Plate Kit Catalog Number K030-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, Urine and Tissue Culture Media

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

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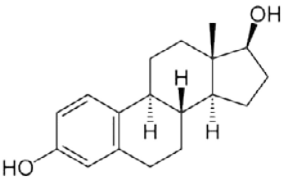
TABLE OF CONTENTS

BACKGROUND

17β-Estradiol, $C_{18}H_{24}O_2$, also known as E2 or oestradiol (1, 3, 5(10)-Estratrien-3, 17β-diol) is a key regulator of growth, differentiation, and function in a wide array of tissues, including the male and female reproductive tracts, mammary gland, brain, skeletal and cardiovascular systems. The predominant biological effects of E2 are mediated through two distinct intracellular receptors, ERα and ERβ, each encoded by unique genes possessing the functional domain characteristics of the steroid/thyroid hormone superfamily of nuclear receptors[1]. ERα is the predominant form expressed in the breast, uterus, cervix, and vagina. ERβ exhibits a more limited pattern and is primarily expressed in the ovary, prostate, testis, spleen, lung, hypothalamus, and thymus[2]. Estradiol also influences bone growth, brain development and maturation, and food intake[3], and it is also critical in maintaining organ functions during severe trauma[4,5]. In plasma, estradiol is bound to serum proteins such as albumin and sex hormone-binding globulin. Just over 2% of E2 is free and biologically active, the percentage remaining constant throughout the menstrual cycle[6]. Estradiol is conjugated in the liver to sulfate and glucuronide derivatives and excreted. Deactivation includes conversion to less-active estrogens, such as estrone and estriol. Estriol is the major urinary metabolite.

Estradiol



1. Giguere, V., Tremblay, A., and Tremblay, GB., “Estrogen receptor beta: re-evaluation of estrogen and antiestrogen signaling”, Steroids, 1998, 63:335–339.
2. Couse, JF., Lindzey, J., Grandien, K., Gustafsson, JA., and Korach, KS., “Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERβ) messenger ribonucleic acid in the wild-type and ERα-knockout mouse.”, Endocrinology, 1997, 138:4613–4621.
3. Butera, PC., “Estradiol and the Control of Food Intake.”, 2010, Physiol. Behav., 99:175-80.
4. Choudhry, MA, and Chaudry, IH, “17-Estradiol: a novel hormone for improving immune and cardiovascular responses following trauma-hemorrhage.”, J. Leuk. Biol., 2008, 83:518-522.
5. Brown, CM, Suzuki, S, Jelks, KAB, and Wise, PM. “Estradiol is a potent protective, restorative, and trophic factor after brain injury.” Semin. Reprod. Med., 2009, 27:240–249.
6. Wu CH, Motohashi T, Abdel-Rahman HA, Flickinger GL, and Mikhail G. “Free and protein-bound plasma estradiol-17 beta during the menstrual cycle.” J. Clin. Endocrinol. Metab., 1976, 43:436–45.

ASSAY PRINCIPLE

The DetectX® Estradiol Immunoassay kit uses a specifically generated antibody to measure estradiol and its metabolites in urine and fecal samples. This kit is not recommended for serum, plasma, or saliva samples as the concentration of estradiol in these samples is too low to be measured without significant concentration. The kit will quantitatively measure Estradiol present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An estradiol 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. An estradiol-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 estradiol to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound estradiol-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 estradiol 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.
Urinary Creatinine Detection Kit (2 or 10 Plate)	K002-H1/H5
Cortisol EIA Kits (Strip Wells)	K003-H1/H5
Cortisol EIA Kits (Whole Plate)	K003-H1W/H5W
Corticosterone EIA Kits	K014-H1/H5
Cortisone CLIA Kits	K017-C1/C5
Cortisone EIA Kits	K017-H1/H5
Progesterone EIA Kits	K025-H1/H5
Estradiol, Serum EIA Kits	KB30-H1/H5
Estrone EIA Kits	K031-H1/H5
PGFM EIA Kits	K022-H1/H5
Ceruloplasmin Activity Kit	K035-H1
Estriol EIA Kits	K064-H1/H5
Estrone-3-Glucuronide (E1G) EIA Kits	K036-H1/H5

SUPPLIED COMPONENTS

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

Kit K030-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

Estradiol Standard
Estradiol at 100,000 pg/mL in a special stabilizing solution.

Kit K030-H1 **or** -H5	125 µL **or** 625 µL	Catalog Number C103-125UL **or** -625UL

DetectX® Estradiol Antibody
A rabbit polyclonal antibody specific for estradiol.

Kit K030-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C101-3ML **or** -13ML

DetectX® Estradiol Conjugate
A estradiol-peroxidase conjugate in a special stabilizing solution.

Kit K030-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C102-3ML **or** -13ML

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

Kit K030-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 K030-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K030-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K030-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K030-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, such as an Eppendorf repeater, with disposable tips to accurately dispense 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 for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Estradiol can be assayed in solid sample types by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols.

Estradiol is identical across all species and we expect this kit to measure estradiol from all sources. The end user should evaluate recoveries of estradiol in other sample matrices being tested.

SAMPLE PREPARATION

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 < 5%.

Urine Samples

Urine samples should be diluted at least 1:4 times with the provided Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated 2 plate and 10 plate Urinary Creatinine Detection kits, K002-H1 and K002-H5.

Tissue Culture Media

For measuring estradiol 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

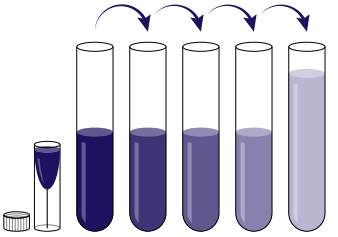
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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

Label test tubes as #1 through #5. Pipet 450 µL of Assay Buffer into tube #1 and 375 µL into tubes #2 to #5. **The estradiol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the estradiol stock solution to tube #1 and vortex completely. Take 125 µL of the estradiol solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #5. The concentration of estradiol in tubes 1 through 5 will be 10,000, 2,500, 625, 156.25, and 39.06 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5
Assay Buffer (µL)	**450**	375	375	375	375
Addition	Stock	Std 1	Std 2	Std 3	Std 4
Vol of Addition (µL)	**50**	125	125	125	125
Final Conc (pg/mL)	10,000	2,500	625	156.25	39.06

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine estradiol 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 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 the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Estradiol Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Estradiol 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 20% 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 estradiol 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-estradiol-eia-kit.assay

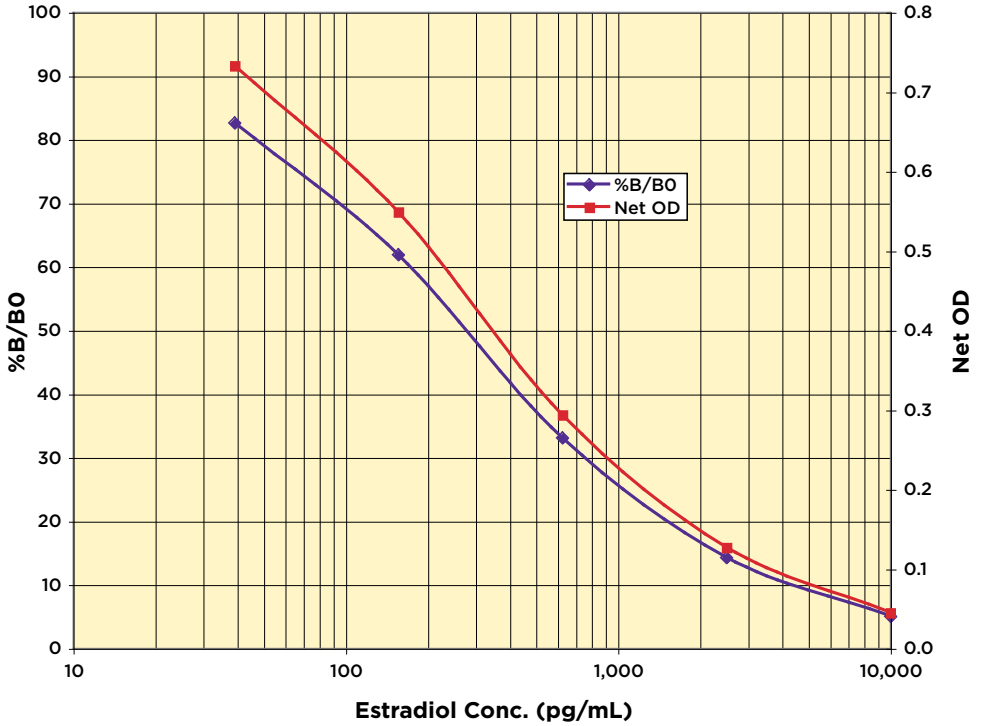
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Estradiol Conc. (pg/mL)
NSB	0.065	0	-	-
Standard 1	0.110	0.045	5.1	10,000
Standard 2	0.192	0.127	14.3	2,500
Standard 3	0.359	0.294	33.1	625
Standard 4	0.614	0.549	61.9	156.25
Standard 5	0.798	0.733	82.6	39.06
B0	0.952	0.887	100.0	0
Sample 1	0.192	0.127	14.3	2,374
Sample 2	0.383	0.318	35.9	556.2

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

Conversion Factor: 100 pg/mL of estradiol is equivalent to 367.1 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

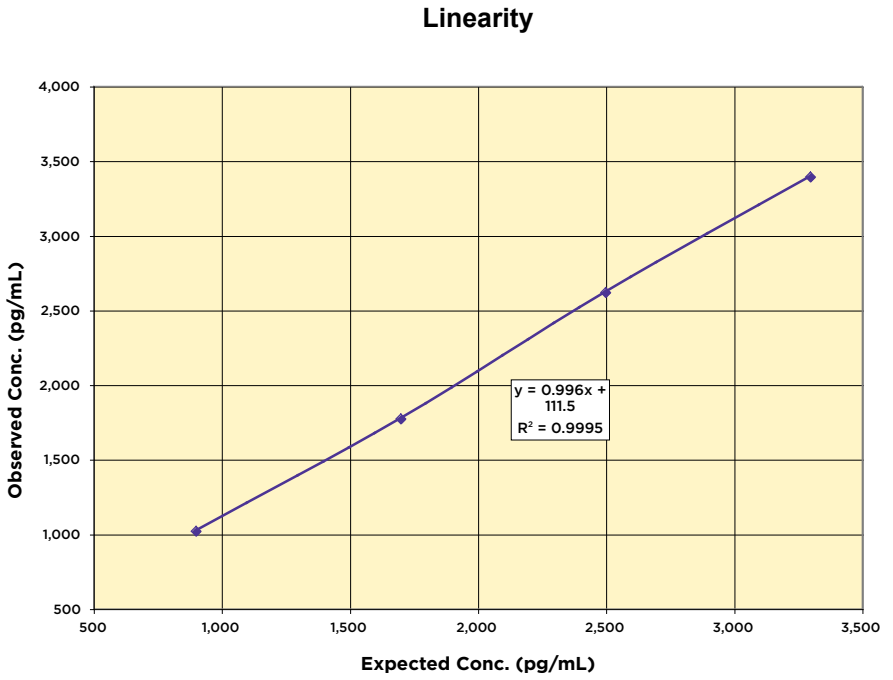
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #5. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 39.6 pg/mL.**

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 sample. **Limit of Detection was determined as 26.5 pg/mL**

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted estradiol level of 99.6 pg/mL and one with a higher diluted level of 4,098 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%	3,393	3,298	102.9
60%	40%	2,620	2,499	104.9
40%	60%	1,772	1,699	104.3
20%	80%	1,021	899	113.6
			Mean Recovery	**106.4%**



Intra Assay Precision

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

Sample	Estradiol Conc. (pg/mL)	%CV
1	2,935	3.9
2	881.3	4.0
3	213.9	7.3

Inter Assay Precision

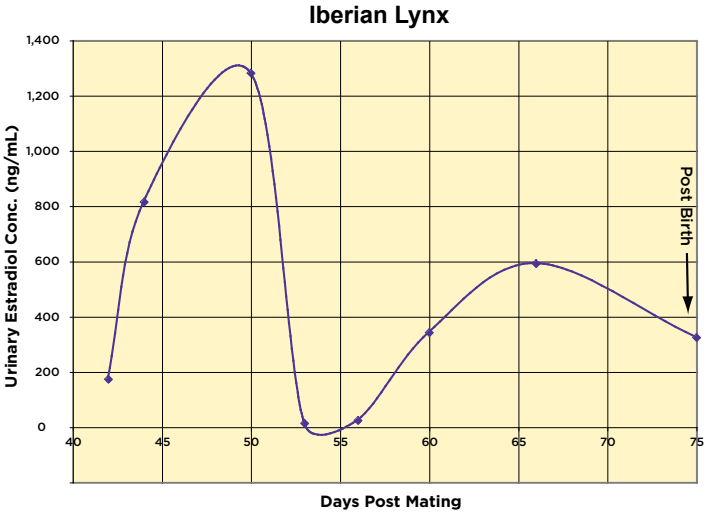
Three human samples were diluted with Assay Buffer and run in duplicates in thirteen assays run over multiple days by four operators. The mean and precision of the calculated Estradiol concentrations were:

Sample	Estradiol Conc. (pg/mL)	%CV
1	2,797	3.6
2	851.2	7.8
3	216.1	13.8

SAMPLE VALUES

Eight human urine samples were tested in the assay, three came from pregnant women who were 10 weeks to 7 months pregnant. Adjusted neat concentrations of Estradiol ranged from 4.041 to 164.1 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine detection kit, K002-H1, the values ranged from 3.57 to 2,240 ng/mg creatinine.

Urine samples from a pregnant Iberian Lynx were tested in the assay.



The Iberian lynx sample was from Martin Dehnhard, Leibniz Institute for Zoo & Wildlife Research, Berlin.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
17β-Estradiol	100%
Estrone	0.78%
17α-Estradiol	0.22%
17α–Ethynylestradiol	0.11%
Estrone Sulfate	< 0.10%
Progesterone	< 0.10%
Testosterone	< 0.10%
5α-dihydroprogesterone	< 0.10%
Cortisol	< 0.10%
Corticosterone	< 0.10%

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

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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 EIA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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

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