

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

Serum 17 β -Estradiol Enzyme Immunoassay Kit

1 Plate Kit Catalog Number KB30-H1

5 Plate Kit Catalog Number KB30-H5

Species Independent

Sample Types Validated:

Multi-Species Serum and Plasma

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

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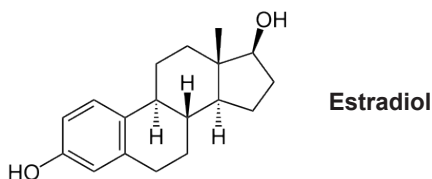
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BACKGROUND

17 β -Estradiol, C₁₈H₂₄O₂, also known as E2 or oestradiol (1, 3, 5(10)-Estratrien-3, 17 β -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, ER α and ER β , each encoded by unique genes possessing the functional domain characteristics of the steroid/thyroid hormone superfamily of nuclear receptors¹. ER α is the predominant form expressed in the breast, uterus, cervix, and vagina. ER β exhibits a more limited pattern and is primarily expressed in the ovary, prostate, testis, spleen, lung, hypothalamus, and thymus². Estradiol also influences bone growth, brain development and maturation, and food intake³, 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 cycle⁶. 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.



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 (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse.", *Endocrinology*, 1997, 138:4613–4621.
3. Butera, PC., "Estradiol and the Control of Food Intake.", *Physiol. Behav.*, 2010, 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[®] Serum Estradiol Immunoassay kit uses a specifically generated antibody to measure estradiol in serum and plasma 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 sheep 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 sheep 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 30 minute 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.
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Cortisol EIA Kits	K003-H1/H5, K003-H1W/H5W
Corticosterone EIA & CLIA Kits	K014-H1/H5, K014-C1/C5
Cortisone EIA & CLIA Kits	K017-H1/H5, K017-C1/C5
Estradiol Non-Invasive EIA Kits	K030-H1/H5
Estrone EIA Kits	K031-H1/H5
Estrone-3-Glucuronide (E1G) EIA Kits	K036-H1/H5
Hemoglobin Dual Range Detection Kit	K013-H1
Progesterone EIA Kits	K025-H1/H5
Urinary Creatinine Detection Kit (2 or 10 Plates)	K002-H1/H5



SUPPLIED COMPONENTS

Coated Clear 96 Well Plate

Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

Kit KB30-H1 or -H5 1 or 5 Each

Catalog Number X061-1EA

Estradiol Standard

Estradiol at 2,400 pg/mL in a special stabilizing solution.

Kit KB30-H1 or -H5 75 μ L or 375 μ L

Catalog Number C158-75UL or -375UL

DetectX[®] Serum Estradiol Antibody

A sheep antibody specific for estradiol.

Kit KB30-H1 or -H5 3 mL or 13 mL

Catalog Number C156-3ML or -13ML

DetectX[®] Serum Estradiol Conjugate

Estradiol-peroxidase conjugate in a special stabilizing solution.

Kit KB30-H1 or -H5 3 mL or 13 mL

Catalog Number C157-3ML or -13ML

Assay Buffer Concentrate

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

Kit KB30-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 KB30-H1 or -H5 30 mL or 125 mL

Catalog Number X007-30ML or -125ML

TMB Substrate

Kit KB30-H1 or -H5 11 mL or 55 mL

Catalog Number X019-11ML or -55ML

Stop Solution

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

Kit KB30-H1 or -H5 5 mL or 25 mL

Catalog Number X020-5ML or -25ML

Plate Sealer

Kit KB30-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 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 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 serum and plasma samples. Samples containing visible particulate should be centrifuged prior to using. 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

Serum and Plasma Samples

The assay has been validated for the measurement of free estradiol in both heparin plasma and serum samples. Serum and plasma samples must be diluted 1:20 or greater with diluted Assay Buffer prior to assaying in the kit. Grossly lipemic or hemolyzed samples should not be used.

Estradiol is typically measured in serum. Heparin plasma samples can also be utilized.

Use all diluted samples within 2 hours of preparation.

Estradiol is produced primarily in ovaries and testes by aromatization of testosterone. Small amounts are produced in the adrenal glands and some peripheral tissues, most notably fat. Most of the circulating estrone is derived from peripheral aromatization of androstenedione. Estradiol and estrone can be converted into each other, and both can be inactivated via hydroxylation and conjugation. Estradiol demonstrates 1.25 to 5 times the biological potency of estrone. Estradiol circulates at 1.5 to 4 times the concentration of estrone in premenopausal, nonpregnant women. Estradiol levels in men and post-menopausal women are much lower than in nonpregnant women, while estrone levels differ less, resulting in a reversal of the premenopausal estradiol:estrone ratio. Estradiol levels in premenopausal women fluctuate during the menstrual cycle. They are lowest during the early follicular phase. Estradiol levels then rise gradually until 2 to 3 days before ovulation, at which stage they start to increase much more rapidly and peak just before the ovulation-inducing lutenizing hormone/folicle stimulating hormone surge at 5 to 10 times the early follicular levels. This is followed by a modest decline during the ovulatory phase. Estradiol levels then increase again gradually until the midpoint of the luteal phase and thereafter decline to trough, early follicular levels.

In human males serum estradiol increases from undetectable levels to about 40 pg/mL by age 18, whereas in females, it increases at puberty and can reach up to 350 pg/mL during menstruation.

Normal adult human male serum estradiol levels range from 10-40 pg/mL. Females levels are typically 15-350 pg/mL, decreasing to less than 10 pg/mL after menopause.

This assay may not be sensitive enough for all serum samples. Please see page 14 for more information on typical concentrations of estradiol and our steroid extraction protocol at: www.arborassays.com/assets/steroid-serum-plasma-extraction-protocol.pdf

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

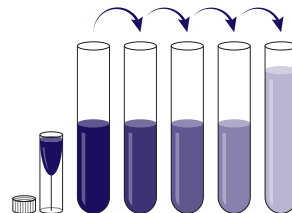
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 #6. Pipet 570 μL of Assay Buffer into tube #1 and 300 μL into tubes #2 to #6. **The estradiol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 30 μL of the estradiol stock solution to tube #1 and vortex completely. Take 300 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 #6. The concentration of estradiol in tubes 1 through 6 will be 120, 60, 30, 15, 7.5 and 3.75 pg/mL .



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (μL)	570	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (μL)	30	300	300	300	300	300
Final Conc (pg/mL)	120	60	30	15	7.5	3.75



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 100 µL of samples or standards into wells in the plate.
3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells).
5. Add 25 µL of the DetectX® Serum Estradiol Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Serum 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. We recommend shaking at around 700–900 rpm. 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-serum-eia-kit.assay

TYPICAL DATA

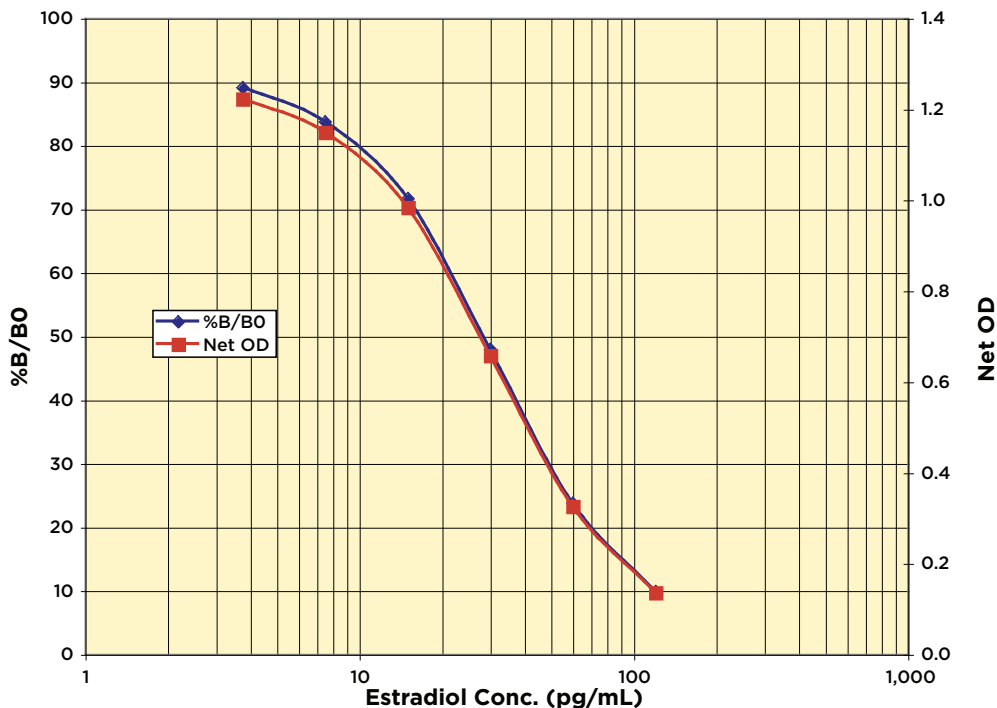
Sample	Mean OD	Net OD	% B/B0	Estradiol Conc. (pg/mL)
NSB	0.096	0	-	-
Standard 1	0.232	0.136	9.9	120
Standard 2	0.422	0.326	23.8	60
Standard 3	0.754	0.658	48.0	30
Standard 4	1.080	0.984	71.7	15
Standard 5	1.245	1.149	83.7	7.5
Standard 6	1.319	1.223	89.1	3.75
B0	1.468	1.372	100	0
Sample 1	1.048	0.952	69.4	16.2
Sample 2	0.382	0.286	20.8	66.8

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 #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 2.21 pg/mL.

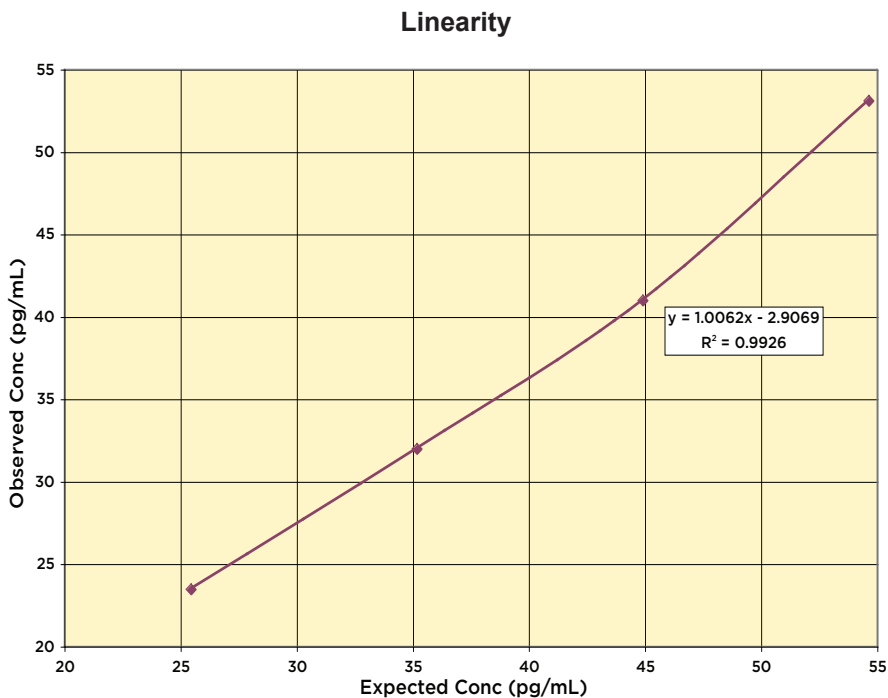
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 2.05 pg/mL

Linearity

Linearity was determined by taking two serum samples diluted with Assay Buffer, one with a low diluted estradiol level of 15.7 pg/mL and one with a higher diluted level of 64.4 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 Serum	Low Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	54.6	53.1	97.2
60%	40%	44.9	41.0	91.3
40%	60%	35.2	32.0	90.9
20%	80%	25.5	23.5	92.2
Mean Recovery				92.9%



Intra Assay Precision

Three human serum 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	58.8	2.4
2	16.1	4.1
3	15.3	5.1

Inter Assay Precision

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

Sample	Estradiol Conc. (pg/mL)	%CV
1	65.9	9.8
2	16.5	9.6
3	16.4	7.4

SAMPLE VALUES

Measurement of serum estradiol forms an integral part of the assessment of reproductive function in females, including assessment of infertility, oligo-amenorrhea, and menopausal status. In addition, it is widely used for monitoring ovulation induction, as well as during preparation for in vitro fertilization. For these applications estradiol measurements with modestly sensitive assays suffice. However, extra sensitive estradiol assays, simultaneous measurement of estrone, or both are needed in a number of other clinical situations. These include inborn errors of sex steroid metabolism, disorders of puberty, estrogen deficiency in men, fracture risk assessment in menopausal women, and increasingly, therapeutic drug monitoring, either in the context of low-dose female hormone replacement therapy or anti-estrogen treatment.

In humans free estradiol levels are between 10-40 pg/mL in normal males, whilst females range from < 10 to > 350 pg/mL dependant on menopausal state. Estradiol levels vary widely during menstrual cycles.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
17 β -Estradiol	100%
Estrone Sulfate	3.20%
Estrone	2.50%
17 α -Ethinyl Estradiol	< 0.3%
Progesterone	< 0.3%
Testosterone	< 0.3%
5 α -dihydroprogesterone	< 0.3%
Cortisol	< 0.3%
Corticosterone	< 0.3%

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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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.

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