Datasheet for ABIN2815092

Estradiol ELISA Kit

1 Image | 2 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	Estradiol
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Estradiol Immunoassay kit uses a specifically generated antibody to measure estradiol and its metabolites in urine and fecal samples.
Brand:	DetectX®
Sample Type:	Fecal, Urine, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	The following cross reactants were tested in the assay and calculated at the 50 % binding point. Steroid Cross Reactivity: Estradiol 100 %, Estrone 0.73 %, Estrone Sulfate < 0.10 %, Progesterone < 0.10 %, Testosterone < 0.10 %, 5a-dihydroprogesterone < 0.10 %, Cortisol < 0.10 %, Corticosterone < 0.10 %
Components:	Coated Clear 96 Well Plates Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG. 1 or 5 Each Estradiol standard Estradiol at 100,000 pg/mL in a special stabilizing solution. 125 µL or 625 µL

Product Details

	DetectX® Estradiol Antibody A rabbit polyclonal antibody specific for estradiol. 3 mL or 13 mL DetectX® Estradiol Conjugate A estradiol-peroxidase conjugate in a special stabilizing solution. 3 mL or 13 mL Assay buffer Concentrate A 5X concentrate that should be diluted with deionized or distilled water. 28 or 55 mL Wash buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL or 125 mL TMB substrate 11 mL or 55 mL Stop solution A 1M solution of hydrochloric acid. CAUstiC. 5 mL or 25 mL Plate sealer 1 or 5 Each
Material not included:	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.

Target Details

Target:	Estradiol
Abstract:	Estradiol Products
Background:	17ß-Estradiol, C H O , also known as E2 or oestradiol (1, 3, 5(10)-Estratrien-3, 17ß-diol) is a key 18 24 2 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, ERa and ERß, each encoded by unique genes possessing the functional domain characteristics of the steroid/thyroid hormone superfamily of nuclear receptors1. ERa is the predominant form expressed in the breast, uterus, cervix, and vagina. ERß 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 trauma4,5. 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

Target Details

	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 (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.", 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. ® www.ArborAssays.com 3

Application Details

Application Notes:	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.
Plate:	Pre-coated
Protocol:	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 450nm 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.
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.
Sample Preparation:	dried Fecal samples: 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.
Assay Procedure:	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 4ºC. 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 wells to act as maximum binding wells (Bo or 0 pg/mL). 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.
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 11.0 10,000 Standard 2 0.192 0.127 27.2 2,500 Standard 3 0.359 0.294 53.6 625 Standard 4 0.614 0.549 77.2 156.25 Standard 5 0.798 0.733 94.4 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. ® 10 EXPECT ASSAY ARTISTRY typical standard Curves 100 0.8 90 0.7 80 0.6 %B/B0 70 Net OD 0.5 60 50 0.4 %B/B0 40 0.3 30 0.2 20 0.1 10 0 0.0 10 100 1,000 10,000 Estradiol Conc. (pg/mL) 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

Application Details

	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
Assay Precision:	Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. 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.
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide
Precaution of Use:	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.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:	Whiting, Ogier, Forbush, Bucko, Gopalan, Seternes, Langeberg, Scott: "AKAP220 manages apical actin networks that coordinate aquaporin-2 location and renal water reabsorption." in:

Publications

Proceedings of the National Academy of Sciences of the United States of America, Vol. 113, Issue 30, pp. E4328-37, (2016) (PubMed).

Images

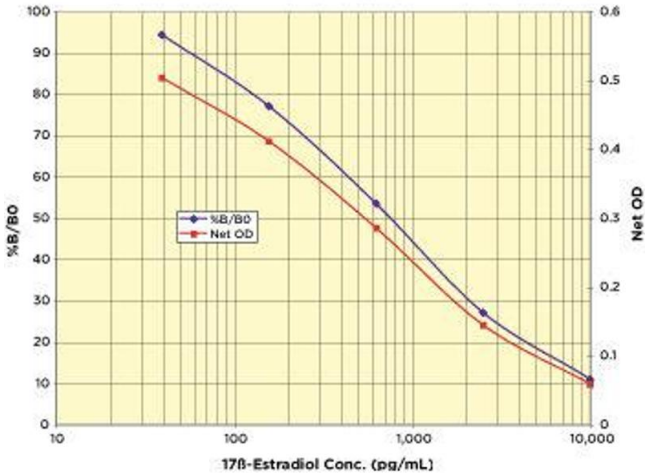

Image 1.