Datasheet for ABIN2866598

Estradiol ELISA Kit

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Overview

Quantity:	96 tests
Target:	Estradiol
Reactivity:	Various Species, Human
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX Serum Estradiol Immunoassay kit is designed to quantitatively measure Free 17ß-estradiol present in serum and plasma samples.
Brand:	DetectX®
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Coated Clear 96 Well Plates Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG. Estradiol Standard Estradiol at 2,400 pg/mL in a special stabilizing solution 75 µL Or 375 µL DetectX® Serum Estradiol Antibody A sheep antibody specific for estradiol 3 mL Or 13 mL DetectX® Serum Estradiol Conjugate 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

Product Details

	TMB Substrate 11 mL Or 55 mL Stop Solution A 1M solution of hydrochloric acid. 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. Contact your plate reader manufacturer for details.

Target Details

Target:	Estradiol
Abstract:	Estradiol Products
Background:	17ß-Estradiol, C18H24O2, also known as E2 or oestradiol (1, 3, 5(10)-Estratrien-3, 17ß-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, 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 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

Target Details

	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 cardio- vascular 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.

Application Details

Application Notes:	This assay has been validated for serum and plasma samples. Samples containing visible particu- late 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.
Plate:	Pre-coated
Protocol:	An estradiol standard is provided to generate a standard curve for the assay and all sam- ples 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 conju- gate. After a short 30 minute incubation, the reaction is stopped and the intensity of the gener- ated 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. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine estradiol 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 deion- ized 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 six 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.
Assay Procedure:	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 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 wells to act as maximum binding wells (B0 or 0 pg/mL). 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. 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.

Application Details

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 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 367.6 pM.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had labo- ratory 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. The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appro- priate precautions when handling this reagent.
Storage:	4 °C,RT
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:

García-Guerra, Canavessi, Jr, Mezera, Sartori, Kirkpatrick, Wiltbank: "Trio, a novel bovine high fecundity allele: III. Acquisition of dominance and ovulatory capacity at a smaller follicle size." in: **Biology of reproduction**, (2018) (PubMed).

Garcia-Guerra, Kamalludin, Kirkpatrick, Wiltbank: "Trio, a bovine high fecundity allele: II. Hormonal profile and follicular dynamics underlying the high ovulation rate." in: **Biology of reproduction**, (2018) (PubMed).

Zena, Dillon, Hunt, Navas, Bícego, Buck: "Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard Salvator merianae." in: **General and comparative endocrinology**, (2018) (PubMed).

Palacios-Arreola, Nava-Castro, Río-Araiza, Pérez-Sánchez, Morales-Montor: "A single neonatal administration of Bisphenol A induces higher tumour weight associated to changes in tumour microenvironment in the adulthood." in: **Scientific reports**, Vol. 7, Issue 1, pp. 10573, (2017) (PubMed).