Datasheet for ABIN2815094

# Estrone ELISA Kit

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## Overview

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

## Product Details

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| Purpose: | The DetectX® Estrone Immunoassay kit is designed to quantitatively measure estrone present in extracted dried fecal samples, urine and tissue culture media 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: Estrone 100 %, Estrone 3-glucuronide 112 %, Estrone 3-sulfate 65.5 %, Estradiol 5.0 %, Estradiol-3-sulfate <0.1 %, Estriol <0.1 %, Progesterone <0.1 %, Pregnandiol <0.1 %, Cortisol < 0.1 %, Androsterone < 0.1 % |
| Components: | Coated Clear 96 Well Plates Clear, break-apart 1 by 8 strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG. 1 Or 5 Each<br>Estrone Standard Estrone at 20,000 pg/mL in a special stabilizing solution. 125 Or 625 µL<br>DetectX® Estrone Antibody A rabbit polyclonal antibody specific for estrone. 3 mL Or 13 mL |

## Product Details

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| | DetectX® Estrone Conjugate A estrone-peroxidase conjugate in a special stabilizing solution. 3 mL Or 13 mL<br>Assay Buffer Concentrate A 5X concentrate that should be diluted with deionized or distilled water. 28 Or 55 mL<br>Wash Buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL Or 125 mL<br>TMB Substrate 11 mL Or 55 mL<br>Stop Solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL Or 25 mL<br>Plate Sealer 1 Or 5 Each |
| Material not included: | Distilled or deionized water.<br>Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL.<br>A microplate shaker.<br>Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.<br>Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.<br>Contact your plate reader manufacturer for de- tails. |

## Target Details

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| Target: | Estrone |
| Abstract: | Estrone Products |
| Target Type: | Amino Acid |
| Background: | Estrone, C18H22O2, also known as E1 or osterone (3-hydroxy-1,3,5(10)-estratrien-17-one) is a C-18 steroid hormone. Estrone is one of the three naturally occurring estrogens, the others being estradiol and estriol1. Estrone is produced primarily from androstenedione originating from the gonads or the adrenal cortex and from estradiol by 17-hydroxysteroid dehydrogenase2. Androstenedione is also converted into estrone by aromatase (CYP19) to estrone and is expressed in stromal and carcinoma or parenchymal components of breast cancer tissue3. Estrone concentrations in premenopausal mammals fluctuate according to the menstrual cycle. In premenopausal women, more than 50 % of the estrone is secreted by the ovaries. In prepubertal children, men and non- supplemented postmenopausal women the major portion of estrone is derived from peripheral tissue conversion of androstenedione. Interconversion of estrone and estradiol also occurs in peripheral tissue. In humans, during the follicular phase of the menstrual cycle estrone levels increase slightly. The production of |

## Target Details

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| | estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 the estrone levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases. Estrone 1. Gruber, CJ, et. al. "Production and actions of estrogens.", N. Engl. J. Med., 2002, 346:340-352. 2. Vance DE., "Cholesterol and related derivatives." In: "Biochemistry", G. Zubay, Ed., 1988, Macmillan Publishing Co., NY, NY, Pgs. 735-748. 3. Miki Y, et al. "Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells.", Cancer Res., 2007, 67:3945-3954. ® www.ArborAssays.com 3 WEB INSERT 150618 |

## Application Details

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| Application Notes: | This assay has been validated for dried fecal, urine and for tissue culture samples.<br>Samples con- taining visible particulate should be centrifuged prior to using.<br>Estrone can be assayed in other sample types by using one of the extraction protocols available on our website at: http://www.arborassays.com/resources/ Estrone is identical across all species and we expect this kit to measure estrone from all sources.<br>The end user should evaluate recoveries of estrone in other sample matrices being tested. |
| Plate: | Pre-coated |
| Protocol: | The kit is unique as it measures both non-conjugated estrone, estrone-3-sulfate and estrone 3-glucuronide in urine and fecal samples with almost equal affinity, allowing for non-invasive testing of this steroid.<br>Please read the complete kit insert before performing this assay.<br>An estrone standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.<br>Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies.<br>An estrone-peroxidase conjugate is added to the standards and samples in the wells.<br>The binding reaction is initiated by the addition of a polyclonal antibody to estrone to each well.<br>After a 2 hour incubation the plate is washed and substrate is added.<br>The substrate reacts with the bound estrone-peroxidase conjugate.<br>After a short incubation, the reac- tion is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength.<br>The concentration of the estrone in the sample is cal- culated, 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. |

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| | We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine estrone concentrations.<br>Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.<br>Assay Buffer Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deion- ized water.<br>Once diluted this is stable at 4 °C for 3 months.<br>Wash Buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water.<br>Once diluted this is stable at room temperature for 3 months.<br>Standard Preparation Label seven test tubes as #1 through #7.<br>Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7.<br>The estrone stock solution contains an organic solvent.<br>Prerinse the pipet tip several times to ensure accurate delivery.<br>Carefully add 50 µL of the estrone stock solution to tube #1 and vortex completely.<br>Take 250 µL of the estrone solution in tube #1 and add it to tube #2 and vortex completely.<br>Repeat the serial dilutions for tubes #3 through #7.<br>The concentration of estrone in tubes 1 through 7 will be 2,000, 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL.<br>Use all Standards within 2 hours of preparation. |
| Sample Preparation: | Dried Fecal Samples :The ethanol concentration in the final Assay Buffer dilu- tion added to the well should be <5 % . Urine Samples Urine samples should be diluted ≥ 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: | 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.<br>2. Pipet 50 µL of samples or standards into wells in the plate.<br>3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.<br>4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).<br>5. Add 25 µL of the DetectX® Estrone Conjugate to each well using a repeater pipet.<br>6. Add 25 µL of the DetectX® Estrone Antibody to each well, except the NSB wells, using a repeater pipet.<br>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 OD |

## Application Details

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| | signal will be approximately 24 % lower. %B/B0 will not be effected.<br>8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.<br>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 concentration for each sample. |
| Calculation of Results: | Average the duplicate OD readings for each standard and sample.<br>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.<br>The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.<br>Or use the online tool from http://www.myassays.com/arbor-assays-estrone-eia-kit.assay to cal- culate the data. *The MyAssays logo is a registered trademark of MyAssays Ltd. typical data Sample Mean OD Net OD % B/B0 Estrone Conc. (pg/mL) NSB 0.048 0 - - Standard 1 0.144 0.096 12.7 2,000 Standard 2 0.192 0.144 19.1 1,000 Standard 3 0.270 0.222 29.5 500 Standard 4 0.353 0.305 40.5 250 Standard 5 0.488 0.440 58.4 125 Standard 6 0.628 0.580 77.0 62.5 Standard 7 0.721 0.673 89.4 31.25 B0 0.801 0.753 100.0 0 Sample 1 0.342 0.294 39.0 283.7 Sample 2 0.611 0.563 74.7 65.6 Always run your own standard curve for calculation of results. Do not use this data.<br>Conversion Factor: 100 pg/mL of estrone is equivalent to 369.9 pM. |
| Assay Precision: | Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay.<br>Inter Assay Precision:<br>Three urine samples were diluted with Assay Buffer and run in duplicates in twelve assays run over multiple days by three operators. |
| Restrictions: | For Research Use only |

## Handling

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| Preservative: | Sodium azide |
| Precaution of Use: | As with all such products, this kit should only be used by qualified personnel who have had labo- ratory safety instruction.<br>The complete insert should be read and understood before attempting to use the product.<br>The antibody coated plate needs to be stored desiccated. |

## Handling

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| | The silica gel pack included in the foil ziploc bag will keep the plate dry.<br>The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.<br>This kit utilizes a peroxidase-based readout system.<br>Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme.<br>Make sure all buffers used for samples are azide free.<br>Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.<br>The Stop Solution is acid.<br>The solution should not come in contact with skin or eyes.<br>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

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| Product cited in: | Leeds, Dennis, Lukas, Stoinski, Willis, Schook: "Biologically validating the measurement of oxytocin in western lowland gorilla (Gorilla gorilla gorilla) urine and saliva using a commercial enzyme immunoassay." in: **Primates; journal of primatology**, (2018) (PubMed).<br><br>Boose, White, Brand, Meinelt, Snodgrass: "Infant handling in bonobos (Pan paniscus): Exploring functional hypotheses and the relationship to oxytocin." in: **Physiology & behavior**, Vol. 193, Issue Pt A, pp. 154-166, (2018) (PubMed).<br><br>Brandtzaeg, Johnsen, Roberg-Larsen, Seip, MacLean, Gesquiere, Leknes, Lundanes, Wilson: " Proteomics tools reveal startlingly high amounts of oxytocin in plasma and serum." in: **Scientific reports**, Vol. 6, pp. 31693, (2016) (PubMed). |

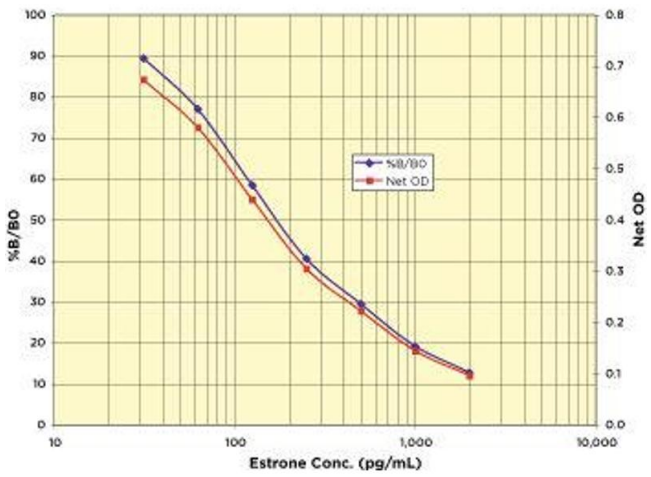
%B/B0
Net OD
Estrone Conc. (pg/mL)
10
100
1,000
10,000
0
10
20
30
40
50
60
70
80
90
100
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

**Image 1.**