Datasheet for ABIN365202

E2 Estradiol ELISA Kit

1 Image | 4 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	E2 Estradiol
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	40-1500 pg/mL
Minimum Detection Limit:	40 pg/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of human estradiol (E2) concentrations in serum, plasma, urine, cell culture supernates, tissue homogenates.
Sample Type:	Serum, Plasma, Cell Culture Supernatant, Urine, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human E2.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	25 pg/mL
Components:	• Assay plate (12 × 8 coated Microwells)

Product Details

	• Standard (freeze dried) • Biotin-antibody (100 × concentrate) • HRP-avidin (100 × concentrate) • Biotin-antibody Diluent • HRP-avidin Diluent • Sample Diluent • Wash Buffer (25 × concentrate) • TMB Substrate • Stop Solution • Adhesive Strip (for 96 wells) • Instruction manual
Material not included:	• Microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 540nm or 570nm. • An incubator which can provide stable incubation conditions up to 37°C ± 0.5°C. • Squirt bottle, manifold dispenser or automated microplate washer. • Absorbent paper for blotting the microtiter plate. • 100mL and 500mL graduated cylinders. • Deionized or distilled water. • Pipettes and pipette tips. • Test tubes for dilution.

Target Details

Target:	E2 Estradiol
Alternative Name:	Estradiol (E2) (E2 Estradiol Products)
Target Type:	Chemical

Application Details

Application Notes:	• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. • Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. • Grossly hemolyzed samples are not suitable for use in this assay. • If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

	• Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals. • Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products. • Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	50 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for E2 and Horseradish Peroxidase (HRP) conjugated E2. The competitive inhibition reaction is launched

	between with HRP labeled E2 and unlabeled E2 with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of E2 in the sample. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	• **HRP-conjugate (1×)** - Centrifuge the vial before opening. HRP-conjugate requires a 100-fold dilution. The suggested dilution is 10µL of HRP-conjugate + 990µL of HRP-conjugate Diluent. • **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×). • **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 400ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (400ng/mL). Sample Diluent serves as the zero standard (0ng/mL).) Note: • Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit. • Bring all reagents to room temperature (18-25°C) before use for 30 min. • Prepare fresh standard for each assay. Use within 4 hours and discard after use. • Making serial dilution in the wells directly is not permitted. • Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL when pipetting. • It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.
Sample Collection:	• **Serum**: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. • **Plasma**: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2-8 °C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. • **Tissue Homogenates**: Rinse 100 mg tissue with 1× PBS, homogenize in 1mL of 1× PBS and store overnight at -20 °C. After two freeze-thaw cycles to break the cell membranes, centrifuge the homogenates for 5 minutes at 5000 × g, 2-8 °C. Remove and assay the supernate immediately. Alternatively, aliquot and store samples at -20 °C or -80 °C.

Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Assay Procedure:

- 1. Prepare all reagents, working standards and samples as directed in the respective sections.
- 2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
- 3. Set a blank well without any solution.
- 4. Add 50µL of standard or sample per well (except blank well). Then add 50µL of HRP-conjugate (1×) to each well (except blank well). Cover the microtiter plate with adhesive strip. Incubate for 30 minutes at 37°C. A plate layout is provided to record standards and samples assayed.
- 5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (200µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher and let it stand for 2 minutes, complete removal of liquid at each stage is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 6. Add 90µL of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light.
- 7. Add 50µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
- 8. Determine the optical density of each well within 5 minutes using a microplate reader set to 450nm. If wavelength correction is available, set to 540nm or 570nm. Subtract readings at 540nm or 570nm from the readings at 450nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450nm without correction may be higher and less accurate.

Note:

- The experiment's final results will be closely related to validity of the products, operation skills of the end users and the environmental conditions.
- Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exced 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between reagent additions. Also, use separate reservoirs for each reagent.
- Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
- Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by

Application Details

	aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision. • Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. • TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light. • Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.
Calculation of Results:	Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Assay Precision:	Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision. Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision. • Intra-assay: CV% less than 8% • Inter-assay: CV% less than 10%
Restrictions:	For Research Use only

Handling

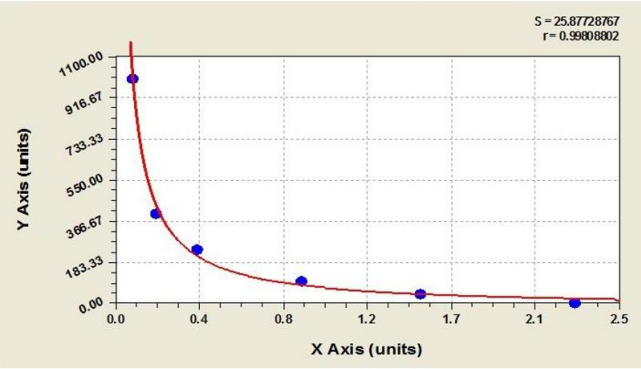
Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing

Handling

	protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Cooke-Hubley, Kirby, Valcour, Mugford, Adachi, Kovacs: "Spine bone mineral density increases after 6 months of exclusive lactation, even in women who keep breastfeeding." in: **Archives of osteoporosis**, Vol. 12, Issue 1, pp. 73, (2018) (PubMed). Lucas, Ramos, Prata, Rodrigues, Costa, Severo, Canhão, Fonseca, Barros: "Changes in serum RANKL and OPG with sexual development and their associations with bone turnover and bone mineral density in a cohort of girls." in: **Clinical biochemistry**, Vol. 47, Issue 12, pp. 1040-6, (2015) (PubMed). Astorino, Harness, Witzke: "Effect of chronic activity-based therapy on bone mineral density and bone turnover in persons with spinal cord injury." in: **European journal of applied physiology**, Vol. 113, Issue 12, pp. 3027-37, (2013) (PubMed).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1.2
1.7
2.1
2.5
X Axis (units)

Image 1.