

Mouse Estradiol (E2) ELISA Kit

Cat. No.: EJ1515562 | Pack size: 48T / 96T | Storage: 2–8°C

Overview

Estradiol (abbreviated E2), also known as follicular hormone or estrin, has the chemical formula $C_{18}H_{24}O_2$ and a molecular weight of 272.38. It exists in two isoforms (α and β) and is the most abundant and biologically potent estrogen. Secreted by granulosa cells of ovarian follicles, its primary metabolites are estrone and estriol. As an 18-carbon steroid, it targets the uterus, vagina, fallopian tubes and pituitary gland. It can be formulated as a transdermal estrogen therapeutic agent. Estrogens stimulate cellular synthesis of DNA, RNA and diverse tissue-specific proteins.

Assay Principle

This kit adopts competitive enzyme-linked immunosorbent assay (competitive ELISA). Microwells pre-coated with mouse estradiol (E2) antigen are sequentially loaded with samples, standards, biotin-conjugated antibody and HRP conjugate, with incubation and washing steps interspersed. Color development is achieved using TMB substrate: TMB turns blue under catalysis of horseradish peroxidase (HRP), and shifts to stable yellow upon acid termination. The color intensity is negatively correlated with the concentration of mouse estradiol (E2) in the sample. Absorbance (OD value) is measured at 450 nm via microplate reader to calculate analyte concentration.

Contents & Storage

Cat. No.	Components	Appearance	48T	96T	Storage
EJ1515562A	Pre-coated Assay Plate	—	48 wells	96 wells	2–8°C
EJ1515562B	Standard	Solid	1 vial	2 vials	2–8°C
EJ1515562C	Universal Diluent	Liquid	1 × 20 mL	2 × 20 mL	2–8°C
EJ1515562D	Biotin-antibody (100×)	Liquid	30 µL	60 µL	2–8°C
EJ1515562E	Streptavidin-HRP (100×)	Liquid	60 µL	120 µL	2–8°C
EJ1515562F	Wash Buffer (20×)	Liquid	1 × 10 mL	2 × 10 mL	2–8°C

Cat. No.	Components	Appearance	48T	96T	Storage
EJ1515562G	TMB Substrate	Liquid	5 mL	10 mL	2–8°C
EJ1515562H	Stop Solution	Liquid	3 mL	6 mL	2–8°C
EJ1515562I	Plate Sealer	—	4 pieces	4 pieces	2–8°C

Note: EJ1515562B, EJ1515562D, EJ1515562E, EJ1515562F shall be diluted in accordance with the instructions.

Storage & Shipping

Storage	Store at 2–8°C
Shipped In	Wet ice
Stability And Storage	Store at 2–8°C long term (6 months).

Precautions

1. Strictly follow the specified incubation time and temperature to guarantee accurate results. All reagents must be equilibrated to room temperature (20–25°C) before use. Refrigerate reagents immediately after use.
2. Improper plate washing may lead to inaccurate readings. Ensure complete removal of residual liquid in wells prior to substrate addition. Avoid prolonged drying of microwells throughout the procedure.
3. Wipe residual liquid and fingerprints from the bottom of the microplate, as contaminants will interfere with OD values.
4. The TMB substrate solution shall remain colorless; discard substrate solution that has turned blue.
5. Prevent cross-contamination between reagents and samples to avoid erroneous test results.
6. Avoid direct intense light exposure during reagent storage and incubation.
7. All reaction reagents must not come into contact with bleach solvents or their volatile fumes; bleach components will destroy the biological activity of kit reagents.
8. Do not use expired products; components from different lot numbers or batch codes cannot be mixed.
9. Recombinant proteins from external sources may fail to be recognized due to mismatched antibody affinity with kit capture antibodies.
10. Handle all potentially infectious samples properly, and dispose of specimens and testing devices in accordance with standardized biosafety protocols.

Sample Handling & Requirements

1. The detectable concentration range of the kit does not equal the analyte concentration range in raw samples. It is recommended to estimate sample analyte concentrations via relevant literature and conduct pre-tests to confirm actual concentrations before formal experiments. Dilute or concentrate samples appropriately if analyte levels fall outside the linear range.
2. If your sample matrix is not listed in this manual, perform a pre-test to verify assay compatibility.
3. Serum: Collect whole blood in serum separation tubes, incubate at room temperature for 2 hours or store overnight at 2–8°C, then centrifuge at 1000×g for 20 minutes. Harvest supernatant for immediate testing, or aliquot and store at –20°C / –80°C; avoid repeated freeze-thaw cycles.
4. Plasma: Collect blood with EDTA or heparin as anticoagulant. Centrifuge specimens within 30 minutes post-collection at 1000×g, 2–8°C for 15 minutes. Collect supernatant for immediate testing, or store at –20°C / –80°C; avoid repeated freeze-thaw cycles.
5. Tissue Homogenate: Rinse tissue with pre-cooled PBS (0.01 M, pH=7.4) to remove residual blood (lysed erythrocytes in homogenate interfere with detection results). Weigh and mince tissue, then mix tissue with PBS at a standard weight-to-volume ratio of 1:9 (e.g., 1 g tissue corresponds to 9 mL PBS; adjust volume as required and record details. Addition of protease inhibitor cocktail to PBS is recommended). Transfer the mixture to a glass homogenizer and grind thoroughly on ice, or use a mechanical homogenizer. For complete cell lysis, sonicate the homogenate or perform repeated freeze-thaw cycles. Finally, centrifuge the homogenate at 5000×g for 5–10 minutes and collect supernatant for testing.
6. Cell Culture Supernatant: Centrifuge culture medium at 1000×g for 20 minutes, collect supernatant for immediate testing, or store at –20°C / –80°C; avoid repeated freeze-thaw cycles.
7. Cell Lysate: For adherent cells, wash gently with pre-cooled PBS, digest with trypsin, then harvest cells by centrifugation at 1000×g for 5 minutes. Suspension cells can be pelleted directly by centrifugation. Wash harvested cells 3 times with pre-cooled PBS, resuspend 1×10^6 cells in 150–200 μ L PBS (protease inhibitors are recommended; reduce PBS volume if analyte abundance is extremely low). Lyse cells via repeated freeze-thaw or sonication. Centrifuge the lysate at 1500×g, 2–8°C for 10 minutes and retain the supernatant for detection.
8. Other Biological Matrices: Centrifuge samples at 1000×g for 20 minutes and collect supernatant for testing.
9. Sample Appearance: Samples must be clear and transparent; all suspended particulates must be removed by centrifugation.
10. Sample Storage: Samples intended for testing within 1 week can be stored at 4°C. For delayed testing, aliquot samples into single-use volumes and freeze at –20°C (stable for 1 month) or –80°C (stable for 6 months). Avoid repeated freeze-thaw cycles. Hemolyzed samples are unsuitable for this assay, as hemolysis will distort final detection results.

Sample Dilution Protocols

Estimate analyte concentration in advance. Follow the dilution schemes below if sample dilution is required:

100-fold dilution (single step): Mix 5 μ L sample with 495 μ L Assay Diluent.

1000-fold dilution (two-step): Step 1: Dilute 5 μ L sample into 95 μ L diluent (20 $\times$). Step 2: Transfer 5 μ L of the 20 $\times$ diluted sample into 245 μ L diluent (50 $\times$), achieving total 1000-fold dilution.

100000-fold dilution (three-step): Step 1: Mix 5 μ L sample with 195 μ L diluent (40 $\times$). Step 2: Transfer 5 μ L 40 $\times$ sample into 245 μ L diluent (50 $\times$). Step 3: Transfer 5 μ L 2000 $\times$ sample into 245 μ L diluent (50 $\times$), total dilution factor = 100000 $\times$.

Guidelines for dilution: Transfer volume per step ≥ 3 μ L; single-step dilution factor $\leq 100\times$. Mix thoroughly after each dilution and avoid bubble formation.

Required Lab Equipment (User-Supplied)

1. Microplate reader (capable of 450 nm absorbance measurement).
2. High-precision pipettes and matching tips: 0.5–10 μ L, 5–50 μ L, 20–200 μ L, 200–1000 μ L.
3. 37°C incubator.
4. Distilled or deionized water.

Pre-Assay Reagent Preparation

1. Remove the kit from refrigeration 10 minutes in advance and equilibrate to room temperature.
2. Serial Standard Working Solution Preparation: Reconstitute lyophilized standard with 1 mL assay diluent, incubate 15 minutes for full dissolution, then mix gently (stock concentration = 50 pg/mL). Prepare serial standards at concentrations: 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0 pg/mL. Serial dilution method: Prepare 7 EP tubes pre-filled with 500 μ L assay diluent each. Transfer 500 μ L 50 pg/mL standard into the first tube to make 25 pg/mL solution, then serially transfer 500 μ L to each subsequent tube for gradient dilution. The final tube (0 pg/mL) serves as blank control with no standard added. The serial dilution procedure is shown in Figure 1.

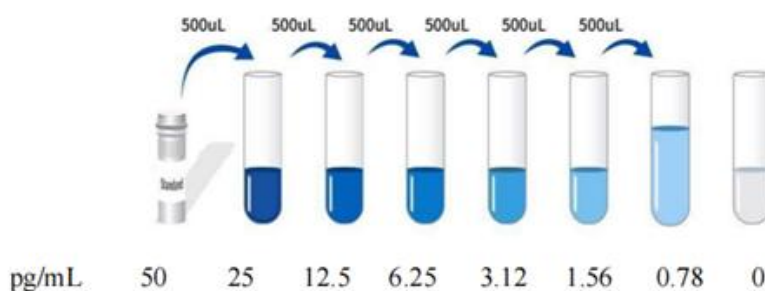


Figure 1. Serial dilution of the mouse E2 standard.

3. Preparation of Biotin-antibody Working Solution: Centrifuge the 100× concentrated Biotin-antibody at 1000×g for 1 minute, 15 minutes before use. Dilute the 100× concentrated Biotin-antibody to 1× working concentration with assay diluent (Example: 10 µL concentrate + 990 µL assay diluent). Prepare fresh right before use.
4. Preparation of Streptavidin-HRP Working Solution: Centrifuge the 100× concentrated Streptavidin-HRP at 1000×g for 1 minute, 15 minutes before use. Dilute the 100× concentrated Streptavidin-HRP to 1× working concentration with assay diluent (Example: 10 µL concentrate + 990 µL assay diluent). Prepare fresh right before use.
5. Preparation of 1× Wash Buffer: Add 10 mL of 20× concentrated wash buffer into 190 mL distilled water. (Crystallization may occur in concentrated wash buffer stored in the refrigerator, which is a normal phenomenon. Place the buffer at room temperature and wait for all crystals to dissolve completely before preparation.)

Assay Procedure

1. Take the required microplate strips from the aluminum foil pouch equilibrated at room temperature for 10 minutes. Seal unused strips in a zip-lock bag and store at 4°C.
2. Sample Loading: Add 50 µL of sample or serially diluted standard to corresponding wells respectively; add 50 µL assay diluent to blank wells. Immediately add 50 µL Biotin-Antibody Working Solution to each well. Seal the plate with sealing film and incubate at 37°C for 60 minutes. (Recommendation: Dilute all test samples at minimum 1× with assay diluent before loading to reduce matrix interference on test results. Multiply the final calculated concentration by the corresponding dilution factor. Technical replicates are recommended for all test samples and standards.)
3. Plate Washing: Discard liquid from wells, add 300 µL 1× Wash Buffer to each well, incubate for 1 minute, flick out buffer and tap the plate dry on absorbent paper. Repeat the washing cycle 3 times (an automated plate washer is acceptable).
4. Streptavidin-HRP Addition: Dispense 100 µL Streptavidin-HRP Working Solution into each well, seal the plate and incubate at 37°C for 30 minutes.
5. Plate Washing: Discard liquid and repeat the washing procedure described in Step 3 for a total of 5 wash cycles.
6. Substrate Development: Add 90 µL TMB substrate to each well, seal the plate and incubate at 37°C in the dark for 15 minutes.
7. Reaction Termination & Reading: Remove the microplate, add 50 µL Stop Solution to every well immediately, then measure absorbance (OD value) of each well at 450 nm without delay.

Data Analysis

1. Calculate the average OD value of replicate wells for standards and samples. Plot a four-parameter logistic standard curve on log-log graph paper with concentration as the X-axis and OD value as the Y-axis (exclude blank well data during plotting).
2. If the sample OD value exceeds the upper limit of the standard curve, dilute the sample appropriately and re-test; multiply the calculated concentration by the corresponding dilution factor for final reporting.

Reference Standard Curve Raw Data (For Illustration Only)

All data and curves below are for reference only; users must generate a standard curve based on their own experimental runs.

Concentration (pg/mL)	50	25	12.5	6.25	3.12	1.56	0.78	0
OD Value	0.169	0.283	0.487	0.908	1.307	1.72	1.945	2.374

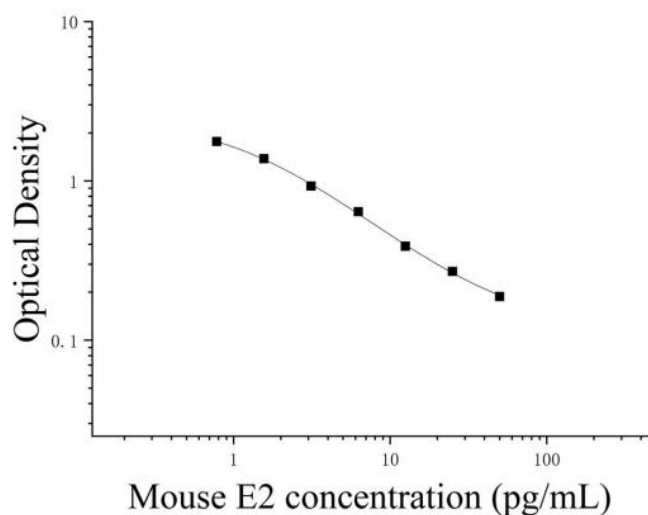


Figure 2. Reference standard curve for mouse E2.

Figure 2 is for reference only; sample concentration shall be calculated based on the standard curve generated from experimental data of each individual test run.

Kit Performance Specifications

1. Precision: Intra-assay coefficient of variation (CV) < 10%, inter-assay CV < 10%.
2. Spike Recovery: Three concentration levels of mouse E2 were spiked into healthy mouse serum, plasma and cell culture supernatant to calculate recovery rates.

Sample Type	Recovery Range (%)	Mean Recovery (%)
Serum (n=8)	81–101	96
Plasma (n=8)	92–105	101
Cell Culture Supernatant (n=8)	97–108	105

3. Linearity upon Serial Dilution: High-concentration mouse E2 was spiked into 4 individual batches of healthy mouse serum, plasma and cell culture supernatant, then serially diluted within the dynamic range of the standard curve to evaluate linearity.

Dilution Ratio	Recovery Metric	Serum	Plasma	Cell Culture Supernatant
1:2	Range (%)	83–95	88–96	90–110
1:2	Mean Recovery (%)	91	93	96
1:4	Range (%)	89–104	87–108	105–115
1:4	Mean Recovery (%)	93	98	110

Troubleshooting Guide

If unsatisfactory assay results occur, take photos of color development, save experimental data, retain used microplate strips and unused reagents, then contact our technical support for troubleshooting. Refer to the troubleshooting items below:

1. Poor standard curve linearity
 - ① Incorrect standard dilution: Reconstitute and dilute standard strictly following recommended protocol.
 - ② Inaccurate pipetting: Calibrate pipettes regularly and verify tip tightness/sealing performance.
 - ③ Evaporation of reaction solution: Seal microplate with plate sealer film.
 - ④ Incomplete washing: Perform sufficient wash cycles with adequate volume of wash buffer.

- ⑤ Foreign residues at well bottom: Wipe microplate bottom prior to absorbance measurement.

2. Faint or absent color development

- ① Insufficient incubation duration: Adhere to specified incubation time.
- ② Incorrect incubation temperature: Incubate at recommended temperature.
- ③ Insufficient reagent addition volume: Verify pipette accuracy and follow operating procedure strictly.
- ④ Improper reagent dilution: Double-check dilution steps for all reagents.
- ⑤ Inactivated Streptavidin-HRP: Mix Streptavidin-HRP with substrate and verify activity via color reaction test.

3. Low OD readings

- ① Wrong microplate reader parameter: Confirm detection wavelength setting on instrument.
- ② Missing stop solution addition: Add appropriate volume of stop solution.
- ③ Overlong delay before plate reading: Measure absorbance immediately after termination.
- ④ Excessively high analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.
- ⑤ Too low analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.

6. High background signal

- ① Contaminated TMB substrate: Replace with fresh substrate solution.
- ② Excess substrate incubation time: Strictly control color development duration.
- ③ Wrong dilution for detection antibody or Streptavidin-HRP: Dilute reagents in accordance with official recommended protocol.
- ④ Incomplete plate washing: Carry out adequate wash cycles with sufficient wash buffer volume.

Recommended Applications

Quantitative determination of mouse estradiol (E2) in serum, plasma, tissue homogenates, cell culture supernatants, cell lysates, and other compatible biological matrices using competitive ELISA.

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- Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.
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