6th Edition, revised in June, 2015

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

E2 (Estradiol)ELISA Kit

Catalog No: E-EL-0065

96T

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

Phone: 86-27-87805095

Email: techsupport@elabscience.com

techelabscience@gmail.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of E2 concentrations in serum, plasma and other biological fluids.

Sensitivity

The minimum detectable dose of E2 is 25pg/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).

Detection Range

40-1400pg/mL

Specificity

No significant cross-reactivity or interference between and analogues was observed.

Note：

Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between E2 and all the analogues.

Repeatability

Coefficient of variation were<15%.

Statement: Thank you for choosing our products. This product is produced by using raw material from world-renowned manufacturer and professional manufacturing technology of ELISA kits. Please read the instructions carefully before use and check all the reagent compositions! If in doubt, please contact Elabscience Biotechnology Co., Ltd.

Storage: All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. Substrate Reagent shouldn't be kept at -20℃ (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.

Kit Components：

Item	Specifications	Storage
Micro ELISA Plate(Dismountable)	12 wells ×8	4℃
Reference Standard (6 tubes)	0.5mL/tube#	4℃
Detection Ab	1vial 6mL	4℃
HRP-labeled Estradiol	1vial 6mL	4℃(shading light)
Concentrated Wash Buffer (20×)	1vial 15mL	4℃
Substrate Reagent A	1vial 7mL	4℃(shading light)
Substrate Reagent B	1vial 7mL	4℃(shading light)
Stop Solution	1vial 7mL	4℃
Plate Sealer	5pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

#: A set of Standard concentrations is 0 pg/mL, 40 pg/mL, 120 pg/mL, 350 pg/mL, 700 pg/mL, 1400 pg/mL.

The reagent in each vial is slightly more that its volume written on lable, please take out the required volume by certain tools, rather than pouring directly.

Test principle

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Goat Anti-Rabbit IgG, make solid-phase secondary antibody. And then add sample, horseradish peroxidase-labeled estradiol and anti-estradiol antibody, so as to form a coated secondary antibody - anti-estradiol antibody - HRP-labeled Estradiol complex. The amount of bound labeled estradiol is inversely proportional to the amount of estradiol in the sample. TMB substrate solution is added to each well after wash the microtiter plate, TMB turn to blue catalyst with horseradish peroxidase (HRP). The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of E2 in the samples is then determined by comparing the OD of the samples to the standard curve.

Sample collection and storage

Samples should be clear and transparent and be centrifuged to remove suspended solids.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8℃. Collect the clear supernate and carry out the assay immediately.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

Sample preparation- Sample should be clear and transparent and be centrifuged to remove suspended solids.

Note:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample preparation

1. Elabscience is only responsible for the kit itself, but not for the samples consumed during the experiment. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the

kit is necessary.

3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected Elisa results due to the impacts of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.
5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. 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.

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Reagent preparation

Bring all reagents to room temperature (18-25℃) before use.

Wash Buffer - Dilute the concentrated wash solution to the working concentration using double distilled water (1:20), mix up. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard –Centrifuge at 1000×g for 1 minute, mix it thoroughly with a pipette.

Washing Procedure:

1. **Automated Washer:** Add 350μL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** Add 350μLWash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

Assay procedure

Bring all reagents and samples to room temperature before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Add Sample:** Take out pre-coated plates. Set a Blank well, Do not add any liquid; Each Standard point set two wells, add 50μl of corresponding Standard per well; 50μl of Sample is added to the rest of each Sample well.
2. Immediately add 50 μl of HRP-labeled Estradiol to each well (except Blank well). Then add 50 μl of Detection Ab to each well. The adding order of Detection Ab should be as the same as the HRP-labeled Estradiol. Thorough mixing, cover with the Plate sealer we provided. Incubate for 1hour at 37℃. (Solutions are added to the bottom of micro ELISA plates well, avoid inside wall touching and foaming as possible.) Mix it gently. To ensure the validity of experimental results, please establish the standard curve for each experiment.
3. **Wash:** Aspirate each well, spin dry and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350μl) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer, soak it for 10 seconds at a time. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
4. **Substrate:** Add 50μl of Substrate Solution A and 50μl of Substrate Solution B to each well. Mix it up. Incubate for about 15 minutes at 37℃. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.
5. **Stop:** Add 50μl of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.
6. **OD Measurement:** Determine the optical density (OD value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.
7. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

Important Note:

1. **Storage:** All the reagents in the kit should be stored following the instructions. Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the tap of reagents should be tightened to prevent evaporation and microbial contamination, or erroneous results may occur.
2. **ELISA Plate:** The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
3. **Add Sample:** The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for

addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.

4. **Incubation:** To prevent evaporation and 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. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
5. **Washing:** The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.
6. **Reaction Time Control:** Please control reaction time strictly following this product description!
7. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.
8. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.
9. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
10. Do not use components from different batches of kit(washing buffer and stop solution can be an exception)
11. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of results

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Troubleshooting

If the results are not good, please take pictures, keep the used plate and remaining reagents. Then contact our technical department. Meanwhile, you could refer to the following materials.

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes and tips
	Insufficiently washed	Ensure washing time, washing times and liquid volume added.
Weak coloration or colorless	Too brief incubation times	Ensure sufficient incubation time;
	Incorrect assay temperature	Use recommended incubation temperature.
	Inadequate reagent volumes	Check pipettes and ensure add all reagents a sufficient amount and in order
	Improper dilution	
Low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution not added	Stop solution should be added to each well before measurement

Declaration:

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Valid period: 6 months.
5. This description is also suitable to 48T kit.