Rat Estradiol ELISA Kit

Enzyme Immunoassay for the quantification of Rat Estradiol ELISA Kit in rat serum.

Catalog number: ARG80755

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Estradiol (E2), also spelled oestradiol, is a steroid, an estrogen, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues such as the breasts, uterus, and vagina during puberty, adulthood, and pregnancy, but it also has important effects in many other tissues, including bone, fat, skin, liver, and the brain. While estrogen levels in men are lower compared to those in women, estrogens have essential functions in men, as well. It is found in most vertebrates and crustaceans, insects, fish, and other animal species.

Estradiol is produced especially within the follicles of the female ovaries, but also in other endocrine (i.e., hormone-producing) and nonendocrine tissues (e.g., including fat, liver, adrenal, breast, and neural tissues). Estradiol is biosynthesized from cholesterol through a series of chemical intermediates. One principal pathway involves the generation of androstenedione, which is converted into estrone by aromatase and then by 17β-hydroxysteroid dehydrogenase into estradiol. Alternatively, androstenedione can be converted into testosterone, an androgen and the primary male sex hormone, which in turn can be aromatized into estradiol. [Info from Wikipedia]

In female rodents, the determination of estradiol is a useful marker in evaluating and monitoring the state of the reproductive functions and pregnancy as well.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Estradiol has been pre-coated onto a microtiter plate. Endogenous Estradiol in samples or standards competes with an Estradiol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of Estradiol in the sample. Estradiol concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 x 8 strips	4°C
Standard 0-5 (0, 5, 20, 80, 320, 1280 pg/ml)	6 vials (lyophilized)	4°C
Incubation Buffer	7 ml (ready to use)	4°C
100X HRP conjugated Estradiol	0.3 ml	4°C
HRP conjugated Estradiol Diluent	30 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml (ready to use)	4°C (Protect from light)
STOP solution	7 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Opened reagents must be stored at 2-8°C. After first opening the reagents are stable for 30 days if used and stored properly.
- Briefly spin down the 100X HRP- Estradiol concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Use reservoirs only for single reagents, especially the reservoirs used for TMB substrate. Using a reservoir for dispensing a TMB substrate solution that had previously been used for the HRP-conjugated Estradiol solution may turn solution colored. Do not pour reagents back into vials as

reagent contamination may occur.

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Note: Any medication containing Estradiol will significantly influence the measurement of Estradiol. This Estradiol ELISA kit should not be used for subjects being treated with the drug fulvestrant (Faslodex®) which cross reacts in the Estradiol ELISA kit and could lead to falsely elevated test results. Lipemic and haemolysed samples can cause false results.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for at least 3 months at room temperature (21-26°C).
- Samples: Samples expected to contain rat Estradiol concentrations higher than the highest calibrator (1280 pg/ml) should be diluted with the Standard zero before assay. The additional dilution step has to be taken into account for the calculation of the results.
- Standards: Reconstitute lyophilized Standards with 0.5 ml distilled water 30 minutes before use. The reconstituted standards can be stored at 2-8°C up to 1 week or -20°C for long term storage.
- 1X HRP-conjugated Estradiol: Immediately before use, dilute HRP-conjugated Estradiol Concentrate 1:100 in HRP-conjugated Estradiol Diluent, mix thoroughly. (e.g. 0.1 ml of concentrated HRP-conjugated Estradiol + 9.9 ml Diluent.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **75 µl** of **standards and samples** in duplicate into appropriate wells.
3. Add **50 µl** of **Incubation Buffer** into each well, mix well. Incubate for **2 hours at room temperature** on a microplate mixer or shaker (600-900 rpm).
4. Immediately before use dilute the HRP-conjugated Estradiol 1:100 in HRP-conjugated Estradiol Diluent.
5. Add **50 µl** of **1X HRP-conjugated Estradiol** into each well, mix well. Incubate for **1 hour at room temperature** on a microplate mixer or shaker (600-900 rpm). (Optimal reaction in this assay is markedly dependent on shaking of the microplate!)
6. Aspirate each well and wash, repeating the process 3 times for a total **4 washes.** Wash by filling each well with **1× Wash Buffer** (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **200 µl** of **TMB substrate** to each well. Incubate for **30 minutes at room temperature** in dark without shaking.
8. Add **50 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at **450 nm** immediately. It is recommended to read the wells within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

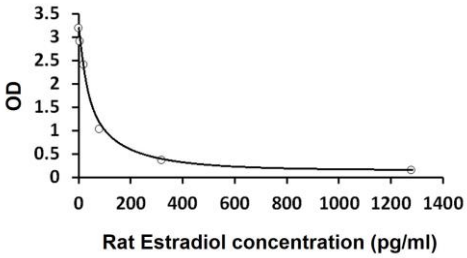
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The concentration of the samples can be determined directly from this calibrator curve.

7. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Specificity

Steroid	Crossreactivity (%)
17-Hydroxyprogesterone	< 0.1
Androstenedione	< 0.1
Corticosterone	< 0.1
E2-3-Glucuronide	3.8
E2-3-Sulphate	3.6
E2-17-Glucuronide	< 0.1
Estriol	0.4
Estradiol	4.2
Fulvestrant	9.5
Pregnenolone	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1

Sensitivity

The lowest analytical detectable level of Estradiol that can be distinguished from the Zero Calibrator is 2.5 pg/ml at the 2SD confidence limit.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.1% and inter-assay precision was 6.03%.

Recovery

81-117%

Linearity

82-117%