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17 beta Estradiol ELISA Kit

A competitive immunoenzymatic assay for the quantitative measurement of 17 beta Estradiol in serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's 17 beta Estradiol in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of 17 beta Estradiol in serum and plasma (citrate).

A 96-well plate has been precoated with anti-Estradiol IgG. Samples and the Estradiol-HRP conjugate are added to the wells, where Estradiol in the sample competes with the added Estradiol-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Estradiol in the sample and the intensity is measured at 450 nm.

Estradiol (17 beta-Estradiol) is a sex hormone. It represents the most predominant Estrogen in women during reproductive years. Estradiol not only has an important impact on reproductive and sexual functioning, but also affects other organs including bone.

During the reproductive years, Estradiol in women is mainly produced by the ovaries, with smaller amounts of Estradiol produced by the adrenal cortex. In men, the testes produce Estradiol.

In plasma, Estradiol is largely bound to sex hormone binding globulin (SHBG) and to albumin; only a fraction is free and biologically active.

Serum Estradiol measurement in women reflects primarily the activity of the ovaries. During Human pregnancy, Estrogen levels, including Estradiol, rise steadily towards term. Estradiol levels increase due to placental production.

In adult premenopausal women, ovarian production of Estradiol is stimulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) during the menstrual cycle. In adult women, Estradiol levels are measured to evaluate fertility and menstrual irregularities and to monitor ovarian follicular function during induction of ovulation. In females, Estradiol acts as a growth hormone for tissue of the reproductive organs. The development of secondary sexual characteristics in women is driven by Estradiol. Estradiol is also involved in male fertility. Estradiol regulates bone maintenance. Post-menopausal women experience an accelerated loss of bone mass due to a relative Estrogen deficiency.

Estradiol affects the production of multiple proteins including lipoproteins, binding proteins and proteins responsible for blood clotting. Estrogens have been found to have neuroprotective function.

Estradiol is involved in some types of cancer such as breast cancer and cancer of the uterine lining. In addition, there are several benign gynaecologic conditions that are dependent on Estrogen such as endometriosis, leiomyomata uteri, and uterine bleeding.

2. Protocol Summary

Prepare all reagents, samples, controls and standards as instructed.

Add samples, standards and controls to wells used.

Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C

After washing, add TMB substrate solution to each well. Incubate at room temperature.

Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-17 beta Estradiol IgG Coated Microplate (12 x 8 wells)	96 wells	4°C
Stop Solution	15 mL	4°C
17 beta Estradiol-HRP Conjugate	22 mL	4°C
TMB Substrate Solution	15 mL	4°C
10X Washing Solution	50 mL	4°C
17 beta Estradiol Control	500 µL	4°C
17 beta Estradiol Standard 0 – 0 pg/mL	1 mL	4°C
17 beta Estradiol Standard 1 – 20 pg/mL	500 µL	4°C
17 beta Estradiol Standard 2 – 120 pg/mL	500 µL	4°C
17 beta Estradiol Standard 3 – 300 pg/mL	500 µL	4°C
17 beta Estradiol Standard 4 – 600 pg/mL	500 µL	4°C
17 beta Estradiol Standard 5 – 2,000 pg/mL	500 µL	4°C
Cover foils	1 unit	4°C
Strip holder	1 unit	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm.
- Incubator at 37°C.
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL.
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer.
- Deionised or (freshly) distilled water.
- Disposable tubes.
- Timer.

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 10X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 50 mL 10X Washing Solution with 450 mL deionized water. Mix thoroughly and gently. Diluted solution is stable for 30 days at 2-8°C. In the concentrated solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals.

All other solutions are supplied ready to use.

10. Sample Preparation

10.1 Plasma and serum:

The determination of 17 beta Estradiol can be performed in plasma as well as in serum. If the assay is performed on the same day as sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 min. before testing.

Avoid repeated freezing and thawing

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

11. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

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12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 25 µL standard, control or sample into their respective wells. Add 200 µL 17 beta Estradiol-HRP Conjugate to each well. Leave a blank well for substrate blank.

12.4 Cover wells with the foil supplied in the kit.

12.5 Incubate for 2 hours at 37°C.

12.6 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL diluted washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 seconds. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

12.7 **Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.**

12.8 Add 100 µL TMB Substrate Solution into all wells.

12.9 Incubate for exactly 30 minutes at room temperature in the dark.

12.10 Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the

microplate gently. Any blue color developed during the incubation turns into yellow.

12.11 Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution.

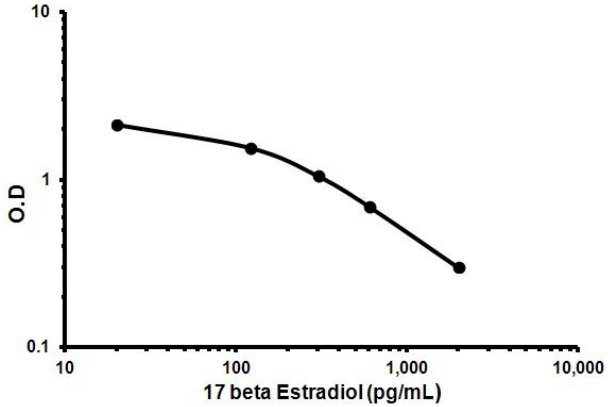
13. Calculations

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D
0	2.43
20	2.11
120	1.54
300	1.05
600	0.69
2,000	0.30

Figure 1. Typical Standard Curve.

15. Typical Sample Values

SENSITIVITY –

The lowest detectable concentration of 17 beta Estradiol calculated subtracting 2X S.D. to the media of ten replicates of standard 0 is 8.68 pg/mL.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
n=	20	3
CV (%)	$\leq$ 9	$\leq$ 10

RECOVERY –

The dilution test conducted with high concentration samples of 17 beta Estradiol gave an average recovery value (± SD) of 95.69% ± 7.74% with reference to the original concentration.

The recovery of 120, 240, 480 and 960 pg/mL of Estradiol added to samples gave an average value (±SD) of 101.09 % ± 5.42 % with reference to the original concentrations.

REFERENCE VALUES –

Human serum Estradiol reference values:

Women	Follicular phase	30 - 100 pg/mL
	Ovulatory peak	130 – 350 pg/mL
	Luteal phase	50 – 180 pg/mL
	Menopause	< 60 pg/mL
Men		< 60 pg/mL
Children		< 40 pg/mL

16. Assay Specificity

The cross reaction of the antibody calculated at 50% is:

Estradiol	100 %
Estrone	2.0 %
Estriol	0.39 %
Fulvestrant	0.09%
Testosterone	0.02 %
Cortisol	7×10^{-3} %
Progesterone	3×10^{-4} %
Dehydroepiandrosterone	1 x 10-4 %

Important note

Fulvestrant is a chemical compound that is found into the formulation of some drugs used in the treatment of some type of cancers in post-menopausal women; due to its chemical similarity with Estradiol, Fulvestrant molecule can interfere with the assay and lead to an over-estimation of Estradiol levels in the sample.

In the case of patients undergoing treatment with Fulvestrant drugs, it is recommended to check the clinical data obtained with the 17 beta-Estradiol kit with other data for Estradiol quantification in order to verify the interference by Fulvestrant.

This method allows the determination of 17 beta Estradiol from 20 – 2,000 pg/mL.

Please contact our Technical Support team for more information.

17. Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4°C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. Notes

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