ab108667

Estradiol 17-beta Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Estradiol 17-beta concentrations in plasma and serum.

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

www.abcam.com
# Table of Contents

1. Introduction ........................................ 2
2. Assay Summary ..................................... 4
3. Kit Contents ....................................... 5
4. Storage and Handling ............................... 6
5. Additional Materials Required .................. 6
6. Preparation of Reagents ........................... 6
7. Preparation and Collection of Specimen ....... 8
8. Assay Method ...................................... 9
9. Data Analysis ...................................... 12
10. Limitations ....................................... 15
11. Specificity ....................................... 16
12. Troubleshooting .................................. 17
1. **Introduction**

ab108667 Competitive immunoenzymatic colorimetric method for quantitative determination of Estradiol 17-beta in human serum or plasma.

Estradiol 17-beta is a sex hormone. It represents the major Estrogen in humans. Estradiol 17-beta has not only a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure. During the reproductive years most Estradiol 17-beta in women is produced by the ovaries, smaller amounts of Estradiol 17-beta are also produced by the adrenal cortex. In men, the testes produce Estradiol 17-beta. In plasma Estradiol 17-beta is largely bound to sex hormone binding globulin (SHBG), also to albumin, only a fraction is free and biologically active. Serum Estradiol 17-beta measurement in women reflects primarily the activity of the ovaries. During pregnancy v levels, including Estradiol 17-beta, rise steadily towards term. Estradiol 17-beta increases due to placental production. In adult premenopausal women, ovarian production of Estradiol 17-beta is stimulated by luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) during the menstrual cycle. In adult women, Estradiol 17-beta levels are measured in the evaluation of fertility and menstrual irregularities, and to monitor ovarian follicular function during induction of ovulation. In the female, Estradiol 17-beta acts as a
growth hormone for tissue of the reproductive organs. The development of secondary sexual characteristics in women is driven by Estradiol 17-beta. Estradiol 17-beta is involved also in men fertility. Estradiol 17-beta regulates the bone maintenance. Post-menopause women experience an accelerated loss of bone mass due to a relative Estradiol 17-beta deficiency. Estradiol 17-beta affects the production of multiple proteins including lipoproteins, binding proteins, and proteins responsible for blood clotting. Estrogens have been found to have neuroprotective function. The Estradiol 17-beta, for his activities, 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 Assay Summary

*ab108667 is based on the principle of a solid phase enzyme-linked immunosorbent assay.*

Microtiter strip wells are precoated with anti-Estradiol 17-Beta antibodies (solid-phase).

\[ \downarrow \]

Estradiol 17-Beta in the sample competes with added horseradish peroxidase labelled Estradiol 17-Beta (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing.

\[ \downarrow \]

The immune complex formed by enzyme-labelled antigen is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of Estradiol 17-Beta in the sample.

\[ \downarrow \]

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.
3 Kit Contents

- Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- Estradiol 17-beta -HRP conjugate: 1 bottle containing 22 ml of horseradish peroxidase labeled Estradiol 17-beta.
- TMB Substrate Solution: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H₂O₂-TMB 0.26g/l) (avoid any skin contact).
- Wash solution 10 x conc.: 1 bottle containing 50 ml of a 10x concentrated solution of phosphate buffer 0.02 M (pH 7.4), NaCl 160 g/l, Tween-20 10 g/l.
- Estradiol 17-beta Standards: 6 bottles, 1 ml of Standard 0, 0.5 ml each of Standard 1 – 5:
  Standard 0: 0 pg/ml
  Standard 1: 20 pg/ml
  Standard 2: 120 pg/ml
  Standard 3: 300 pg/ml
  Standard 4: 600 pg/ml
  Standard 5: 2000 pg/ml
- 1 Strip holder
- 1 Cover foil
4 Storage and Handling

The reagents are stable up to the expiry date stated on the label when stored at 2-8 °C in the dark.

5 Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
- Disposable tubes
- Microtiter well reader capable of reading absorbance at 450 nm.
- Vortex mixer, or equivalent
- Manual or automatic equipment for rinsing wells
- Timer
- Incubator 37°C

6 Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. Coated snap-off Strips: The ready to use break apart snap-off strips are coated with anti-Estradiol 17-beta IgG antibodies. Store at 2-8 °C. Open the bag only when it is at room
temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

3. **Estradiol 17-beta -HRP Conjugate:** The conjugate is ready to use. Mix gently on a rotating mixer for 5 min. After first opening it is stable for another 6 months if stored at 2-8°C.

4. **Estradiol 17-beta Standards:** The standard solutions are ready to use. *After first use the standards are still stable for another 6 months if stored at 2-8 °C.*

5. **TMB Substrate Solution:** The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

6. **Stop Solution:** The bottle contains 15 ml 0.15 M sulphuric acid solution. This ready to use solution has to be stored at 2-8°C.

7. **Wash Solution:** Dilute the concentrated solution with distilled water to a final volume of 500 ml prior to use. For smaller volumes respect the 1:10 dilution ratio. The 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. For greater accuracy dilute the whole bottle of concentrated wash
solution to 500 ml and take care that all crystals are transferred by washing the bottle, then mix until crystals are completely dissolved.

7 Preparation and Collection of Specimen

The determination of Estradiol can be performed in plasma as well as in serum. If the assay is performed on the same day of sample collection, the specimen should be kept at 2...8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples gently for 5 min. before testing. Avoid repeated freezing and thawing.

Precaution:

- The reagents contain Proclin 300® as preservative
- Do not use heavily haemolysed samples.
- Maximum precision is required for dilution and dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- This method allows the determination of Estradiol from 20 pg/ml to 2000 pg/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair Estradiol determination.
8. Assay Method

Test Preparation:

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank

2 wells (e.g. B1+C1) for standard 0

2 wells (e.g. D1+E1) for standard 1

2 wells (e.g. F1+G1) for standard 2

2 wells (e.g. H1+A2) for standard 3

2 wells (e.g. B2+C2) for standard 4

2 wells (e.g. D2+E2) for standard 5

- *It is recommended to determine standards and samples in duplicate.*
- Perform all assay steps in the order given and without any appreciable delays between the steps.
• A clean, disposable tip should be used for dispensing each standard and each sample.
• Adjust the incubator to 37 °C.

Assay Procedure:

1. Dispense 25 μl standards and samples into their respective wells. Add 200 μl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 2 hour at 37°C.**
4. 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 >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

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

5. Dispense 100 μl TMB Substrate Solution into all wells.
6. **Incubate for exactly 30 min at room temperature (22-28°C) in the dark.**
7. Dispense 100 μl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
Any blue colour developed during the incubation turns into yellow.

8. Measure the absorbance (E) of the specimen at 450 nm within 30 min after addition of the Stop Solution.

**Measurement:**

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and sample in the distribution and identification plan.

Where applicable calculate the mean absorbance values of all duplicates.

**Quality Control:**

Each laboratory should assay controls at normal, high and low levels range of Estradiol 17-beta for monitoring assay performance. These controls should be treated as unknowns and values determined in
every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations. If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

9. Data Analysis

1. Calculate the mean absorbance for each point of the standard curve and each sample.
2. Plot the mean value of absorbance of the standards against concentration.
3. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).
4. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/ml.

A. Reference values

The serum Estradiol 17-beta reference values (pg/ml):

<table>
<thead>
<tr>
<th>Woman</th>
<th>Follicular phase</th>
<th>30-100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovulatory peak</td>
<td>130-350</td>
</tr>
<tr>
<td></td>
<td>Luteinic phase</td>
<td>50-180</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>&lt;60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Man</th>
<th>&lt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>

B. Sensitivity

The lowest detectable concentration of Estradiol 17-beta calculated subtracting 2x S.D. to the media of ten replicates of standard 0 is 8.68 pg/ml.
C. Recovery

The dilution test conducted with high concentration samples of Estradiol 17-beta gave an average recovery value (± SD) of 95.69% ± 7.74% with reference to the original concentration. The recovery of 120 – 240 - 480 – 960 pg/ml of Estradiol 17-beta added to samples gave an average value (±SD) of 101.09 % ± 5.42 % with reference to the original concentrations.

D. Standardisation

ab108667 was compared to another commercially available Estradiol 17-beta assay. 16 serum samples were analysed in both test systems. The linear regression curve was calculated:

\[(ab108667) = 1.03 \times (\text{Estradiol 17-beta Reference}) - 12.96\]

\[r^2 = 0.996\]

E. Reproducibility

Intra Assay Variation: Within run variation was determined by replicate determination (10x) of two different control sera in one assay. The within assay variability is ≤ 9%.
Inter Assay Variation: Between run variation was determined by replicate measurements of three different human sera in different lots. The between assay variability is \( \leq 10\% \).

**10. Limitations**

- Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. 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 one plate is used, it is recommended to repeat the dose response curve.

- Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.

- Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
The cross reaction of the antibody calculated at 50% according to Abraham:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol 17-beta</td>
<td>100%</td>
</tr>
<tr>
<td>Estrone</td>
<td>2%</td>
</tr>
<tr>
<td>Estriol</td>
<td>0.39%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.2%</td>
</tr>
<tr>
<td>Cortisol</td>
<td>$7 \times 10^{-3}$%</td>
</tr>
<tr>
<td>Protgesterone</td>
<td>$3 \times 10^{-4}$%</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>$1 \times 10^{-4}$%</td>
</tr>
</tbody>
</table>
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Condition/Issue</td>
<td>Possible Solution</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed: Wash wells as per protocol recommendations</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit: Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
<td></td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in Japan
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888

Copyright © 2011 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.