ab108667 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.

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.

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	500 µL	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

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to 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.

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.

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.

Sample Preparation

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.

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).

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.

1. Prepare reagents, standards, and samples as directed in the previous sections.
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.
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.
4. Cover wells with the foil supplied in the kit.
5. Incubate for 2 hours at 37°C.
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.
7. **Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.**
8. Add 100 µL TMB Substrate Solution into all wells.
9. Incubate for exactly 30 minutes at room temperature in the dark.
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.
11. Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution

Calculations

1. 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).
2. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

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 (%)	≤ 9	≤ 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

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.

Typical standard data 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

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)
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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)