

ab178662 – Estriol ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of estriol in saliva.

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

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1. BACKGROUND

Abcam's Estriol *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of estriol in saliva.

A 96-well plate has been precoated with anti-estriol antibodies. Samples and the estriol HRP Conjugate are added to the wells, where estriol in the sample competes with the added estriol HRP Conjugate 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 estriol in the sample and the intensity is measured at 450 nm.

Estriol (also Oestriol) is one of the three main Estrogens' produced by the human body. It is only produced in significant amounts during pregnancy as it is made by the fetus.

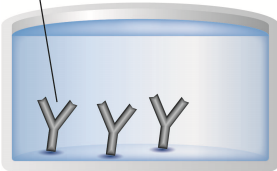
During pregnancy the production of estriol depends on an intact maternal-placental-fetal unit. Fetal-placental production of estriol leads to a progressive rise in maternal circulating levels reaching a late-gestational peak several orders of magnitude greater than non-pregnant levels. In the maternal circulation, estriol undergoes a rapid conjugation in the liver followed by urinary excretion with a half-life of about 20 minutes. Since normal estriol production depends on an intact maternal-placental-fetal circulation and functional fetal metabolism, maternal estriol levels have been used to monitor fetal status during pregnancy, particularly during the third trimester.

DHEA is produced by the adrenal cortex of the fetus, this is converted to estriol by the placenta. If levels are abnormally low in a pregnant woman, this may indicate a problem with the development in the child.

Levels of estriol in non-pregnant women do not change much after Menopause, and levels are not significantly different from levels in men.

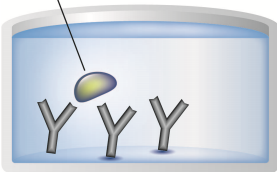
2. ASSAY SUMMARY

Capture Antibody



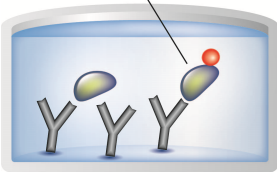
Prepare all reagents, samples, controls and standards as instructed.

Sample



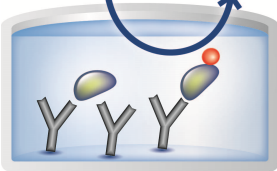
Add samples, standards and controls to wells used.

Labeled HRP-Conjugate



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

Substrate Colored Product



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
anti-Estriol IgG Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
Estriol HRP Conjugate	1 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
50X Washing Solution	20 mL	2-8°C
Estriol Low Control	1 mL	2-8°C
Estriol Medium Control	1 mL	2-8°C
Estriol Standard 0 - 0 pg/mL	1 mL	2-8°C
Estriol Standard 1 - 2.5 pg/mL	1 mL	2-8°C
Estriol Standard 2 - 15 pg/mL	1 mL	2-8°C
Estriol Standard 3 - 100 pg/mL	1 mL	2-8°C
Estriol Standard 4 - 600 pg/mL	1 mL	2-8°C
Estriol Standard 5 - 4,000 pg/mL	1 mL	2-8°C
Incubation Buffer	30 mL	2-8°C
Strip Holder	1 Unit	2-8°C
Cover Foils	1 Unit	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 nm or 620-630 nm
- 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

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

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, samples and controls to room temperature (18-25°C) prior to use. At the end of the assay, store reagents immediately at 2-8°C; avoid long exposure to room temperature.

9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 1,000 mL 1X Washing Solution combine 20 mL 50X Washing Solution with 980 mL deionized water. Mix thoroughly and gently. Diluted solution is stable for 30 days at 2-8°C.

9.2 Estriol HRP Conjugate

Prepare immediately before use. Add 10 µL of Estriol HRP Conjugate to 1 mL Incubation Buffer. Mix thoroughly and gently. Diluted solution is stable for 3 hours at room temperature.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

The determination of estriol can be performed in saliva. It is recommended to collect saliva samples commercially available sample collector.

If no specific instructions have been given, saliva samples may be collected at any time, paying attention to the following indications:

- 10.1 Let the saliva flow down through the straw into the centrifuge glass tube
- 10.2 Centrifuge the sample for 15 minutes at 3,000 rpm
- 10.3 Store at -20°C for at least 1 hour
- 10.4 Defrost samples
- 10.5 Centrifuge again for 15 minutes at 3,000 rpm
- 10.6 The saliva sample is now ready to be tested
- 10.7 The specimen should be kept at 2-8°C for up to one week; otherwise it should be aliquoted and stored deep-frozen (-20°C).

Notes If saliva collection has to be carried out in the morning ensure that this is carried out prior to brushing teeth.

During the day saliva should be collected at least 1 hour after any food or drink.

It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other such extraneous materials.

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).

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 μ L to 350 μ L to avoid washing effects.**
- **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 50 μ L standard, control or sample into their respective wells. Add 100 μ L estriol 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 1 hour at room temperature.
 - 12.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μ L 1X Washing Solution. Avoid overflows from the reaction wells. During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.
 - 12.7. Add 100 μ L TMB Substrate Solution into all wells.
 - 12.8. Incubate for exactly 15 minutes at room temperature in the dark.

ASSAY PROCEDURE

- 12.9. Add 100 μ L 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.
- 12.10. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm or against the blank within 5 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 SAMPLE VALUES

REFERENCE VALUES-

As the values of salivary estriol have a circadian pattern we suggest collecting the samples at the same hour/

The following values can be used as preliminary guideline until each laboratory has established its own normal range.

Group	Time	n	Median ± SD (pg/mL)	Absolute Range (pg/mL)
Women	8 AM	21	0 – 21.0	0 – 32.0
Premenopausal	5 AM	21	0 – 6.8	0 – 8.9

Pregnancy	Saliva (pg/mL)
22 Weeks	700 ± 500
24 Weeks	900 ± 600
26 Weeks	1200 ± 700
28 Weeks	1500 ± 800
30 Weeks	1800 ± 800
34 Weeks	2200 ± 1,100
36 Weeks	3200 ± 1,300
37 Weeks	4100 ± 1,600
38 Weeks	4500 ± 1,700
39 Weeks	5000 ± 2,000
40 Weeks	5700 ± 2,000

SENSITIVITY –

The lowest detectable concentration of estriol that can be distinguished from standard 0 is 1.0 pg/mL at the 95% confidence limit.

PRECISION –

	Intra-Assay	Inter-Assay
n=	16	10
%CV	≤ 9.7%	≤ 13.7%

RECOVERY –

The recovery of 50 – 300 – 2,000 pg/mL estriol added to a sample of “saliva-free” sample gave an average value (\pm SD) of 104% \pm 6.0% with reference to the original concentrations.

15. ASSAY SPECIFICITY

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

Estriol saliva	100%
16-epi-estriol	10.5%
15 α -OH-estriol	7.0%
Estriol-3-sulphate	2.0%
Estradiol	0.1%
17-epi-estriol	$<1 \times 10^{-2}$ %
Estriol-3 α glucuronate	$<1 \times 10^{-2}$ %
Estriol- 16 α glucuronate	$<1 \times 10^{-2}$ %
Estrone	$<1 \times 10^{-4}$ %

16. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too 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/thaw cycles)

RESOURCES

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

17. NOTES

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