Estriol

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55113-96

www.zellx.de

Sample Types Validated for:

Serum and Plasma, Urine, saliva, Dried Fecal Extracts, and Tissue Culture Media

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

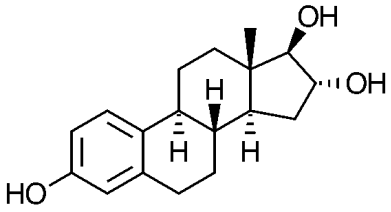
Please read this insert completely prior to using the product.

Introduction

Background

Estriol ($C_{18}H_{24}O_3$), also known as E3 or oestriol, is a C-18 steroid hormone involved in female sexual development and function. It is one of the three endogenous Estrogens in woman, the two others are Estradiol and Estrone. Being important for the health of the mother and baby, Estriol constitutes 60-70% of the total Estrogens during pregnancy. It is mainly produced by placenta in pregnant women, increasing to 300-500-fold higher than in non-pregnant women. Approximately 90% of the precursors for the formation of Estriol are of fetal origin; the late term human fetus produces relatively large amounts of 16α-hydroxy DHEA, which serves the mother as a precursor of Estriol. In case of abnormal maternal serum screening results, specifically low levels of unconjugated Estriol in the second trimester, a diagnosis of Smith-Lemli-Opitz syndrome (SLOS) may be suspected. SLOS is an autosomal recessive disorder caused by mutations of the gene encoding 7-dehydrocholesterol reductase.

Regarding the biochemistry of Estriol, it is a hydroxylated metabolite of 17β-Estradiol or Estrone with a hydroxyl group at the C3, 16α, and 17β positions. As a metabolite of Estrone, it is metabolized via 16α-hydroxyestrone through the enzyme 16α-hydroxysteroid dehydrogenase or to 2- or 4-hydroxyestrone by the action of catecho-O-methyltransferase. The latter metabolites can be formed in the brain and may compete with receptors for catecholamines. Estriol is also a major urinary Estrogen whose metabolites are conjugated with sulfate or glucuronide prior to excretion by the kidney.



Assay principle

The ZellX® Estriol Immunoassay kit is a competitive ELISA assay designed to quantitatively measure Estriol present in urine, saliva, extracted serum and plasma, extracted dried fecal samples, and tissue culture media**.** An Estriol stock solution is provided to generate a standard curve for the assay, and all samples should be read off the standard curve.

The kit includes a 96-well plate that has been pre-coated with a secondary goat anti-rabbit antibody. The function of this antibody is to capture the rabbit anti-Estriol antibody bound to Estriol conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The Estriol-conjugate (labeled) and the sample Estriol (unlabeled) compete for binding to the rabbit antibody. After 2 hours of incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of Estriol in the sample, the stronger the signal is, due to more labeled Estriol bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Estriol Standard (120 ng/mL)	125 µL
Estriol Antibody	2.6 mL
Estriol Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents, except the Estriol Conjugate, should be stored at 4° C until the expiration date of the kit.
The Estriol Conjugate must be stored at -20° C.

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, and Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For Dried Fecal Samples:

ACS Grade Ethanol

Glass test tubes

For Serum and Plasma Samples:

Diethyl ether or ethyl acetate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The color of silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Wash Buffer can be stored at room temperature for up to 3 months.

Sample preparation

This assay has been validated for extracted serum and plasma, urine, saliva, dried fecal, and tissue culture media samples. This kit is not recommended for serum or plasma samples without extraction. Samples containing visible particulate should be centrifuged prior to use. Estriol can be also assayed in other sample types; for sample preparation method please contact us at technical@zellx.de.

Since Estriol is identical across all species, it is expected that this kit can measure Estriol in human and other species.

All samples and standards must be used within 2 hours of preparation.

I. **Serum & Plasma:**

- Use our detailed extraction protocol for Steroid Liquid Extraction at https://zellx.de/wp-content/uploads/2020/07/Steroid-Liquid-Extraction-Protocol.pdf.
- **We strongly recommend using diethyl ether or ethyl acetate for extraction.**

II. **Urine:**

- Urine should be diluted ≥ 1:8 by taking one part of sample and adding 7 or more parts of Assay Buffer prior to conducting the assay.
  Normalize the sample value based on Creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.

III. **Saliva:**

- Freeze the collected saliva at -20°C.
- Upon thawing, centrifuge it at 2500 g for 20 minutes and collect the clear supernatant. Analyze collected supernatant immediately or aliquot and freeze at -20°C.
- Saliva should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting the assay.

IV. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60 °C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

V. **Tissue Culture Media:**

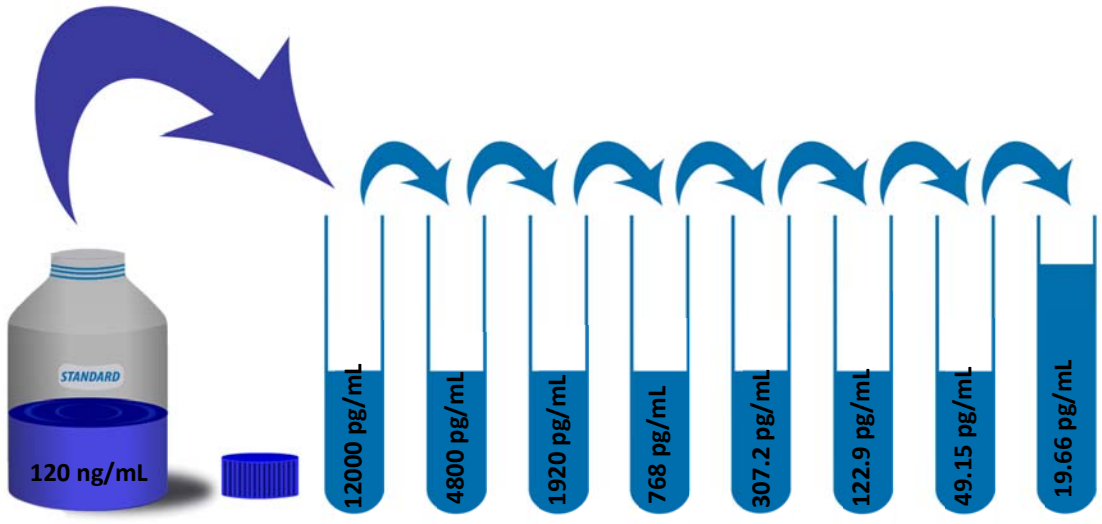
- For measuring Estriol in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All the samples must be used within 2 hours of preparation.

Standard preparation

- Prepare a 1:10 dilution of Estriol Standard with Assay Buffer (mix 40 µL of standard with 360 µL of Assay Buffer), and label as the Standard No.8 (12000 pg/mL).
- The Estriol Standard contains an organic solvent. Prerinse the pipette tip several times to ensure accurate volume is delivered.
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.8	12000 pg/mL	40 µL Estriol Standard + 360 µL Assay Buffer
Standard No.7	4800 pg/mL	200 µL Standard No.8 + 300 µL Assay Buffer
Standard No.6	1920 pg/mL	200 µL Standard No.7 + 300 µL Assay Buffer
Standard No.5	768 pg/mL	200 µL Standard No.6 + 300 µL Assay Buffer
Standard No.4	307.2 pg/mL	200 µL Standard No.5 + 300 µL Assay Buffer
Standard No.3	122.9 pg/mL	200 µL Standard No.4 + 300 µL Assay Buffer
Standard No.2	49.15 pg/mL	200 µL Standard No.3 + 300 µL Assay Buffer
Standard No.1	19.66 pg/mL	200 µL Standard No.2 + 300 µL Assay Buffer
Standard No.0	0 pg/mL	300 µL Assay Buffer



All standard must be used within 2 hours of preparation

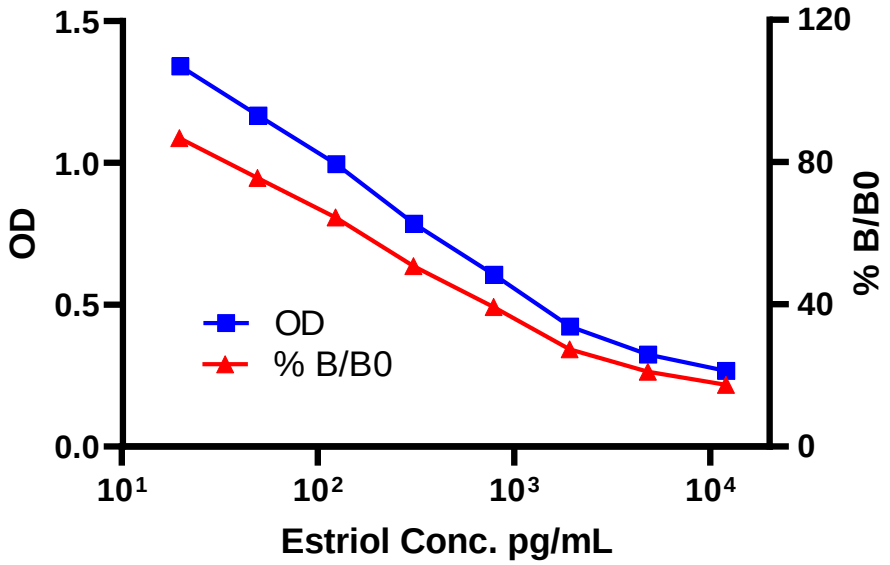
Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 75 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Estriol Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Estriol Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 2 hours at room temperature. If the plate is not shaken, signals will be approximately 30 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes without shaking.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 pg/mL of Estriol is equivalent to 346.8 pM



A typical standard curve of ZellX® Estriol ELISA kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Estriol ELISA kit was determined as 6.81 pg/mL.

Sensitivity

The sensitivity of the ZellX® Estriol ELISA kit was determined as 5.33 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 5 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 5 human samples were tested in duplicate on 12 different assays over multiple days.

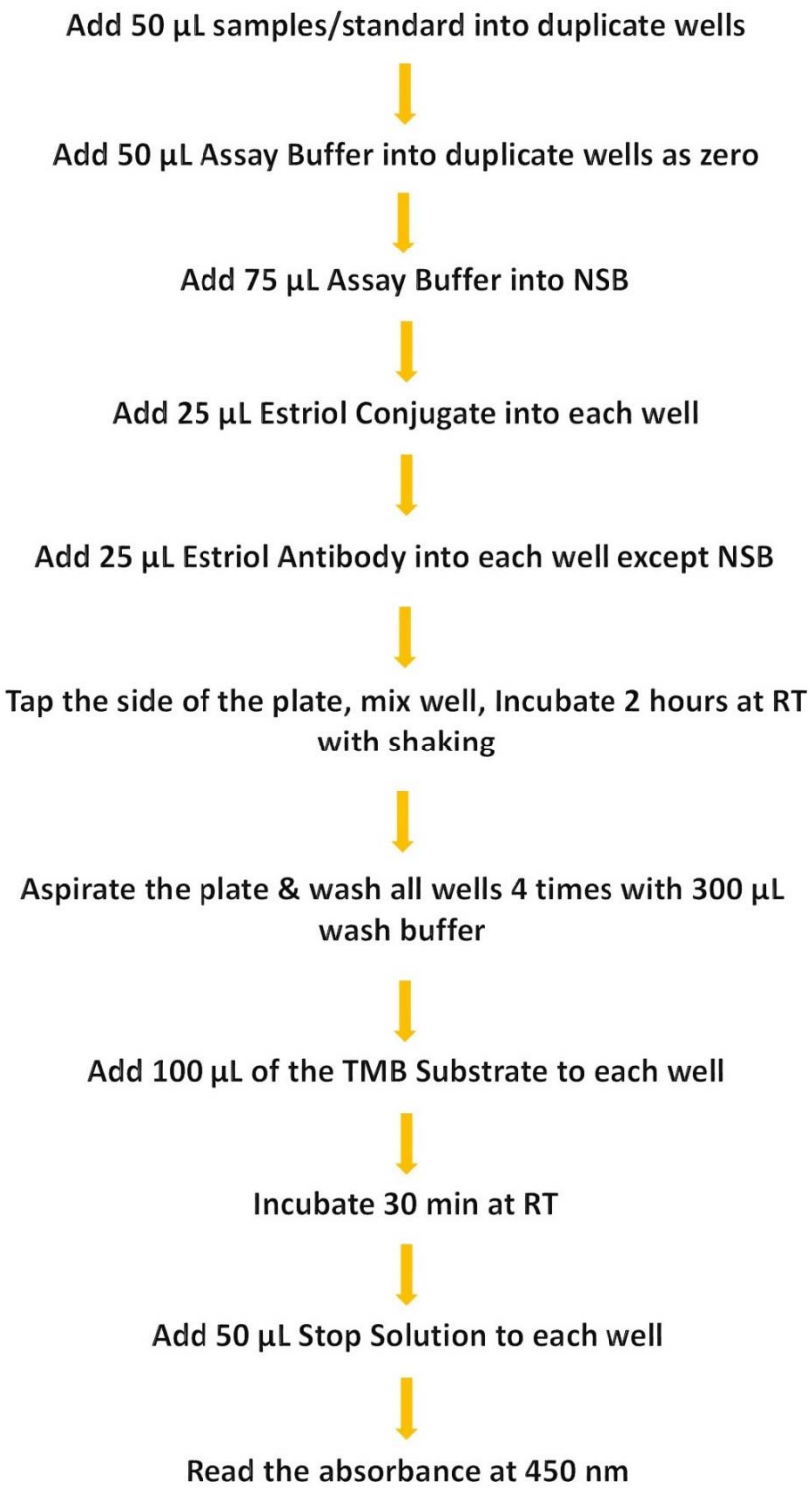
Item	*% CV*
Intra assay	8.4, 13.1, 11.3, 8.3, 8.4
Inter assay	10.5, 11.0, 10.1, 17.5, 11.6

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50 % binding point.

Steroid	*Cross Reactivity (%)*
Estriol	100
Estriol 3-glucuronide	57.16
Estriol 3-sulfate	38.5
16-Epiestriol	6.77
17β-Estradiol	0.03
17-Epiestriol	0.02
Androstenedione	< 0.01
Androsterone	< 0.01
Corticosterone	< 0.01
Cortisol	< 0.01
Cortisone	< 0.01
Desoxycorticosterone	< 0.01
DHEA	< 0.01
DHEA-S	< 0.01
DHT	< 0.01
17α-Estradiol	< 0.01
Estrone	< 0.01
Ethynylestradiol	< 0.01
Progesterone	< 0.01
Testosterone	< 0.01

Protocol summary



References

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