High sensitivity Estradiol

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55108-96

www.zellx.de

Sample Types Validated for:

Serum and Plasma

!!! 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.

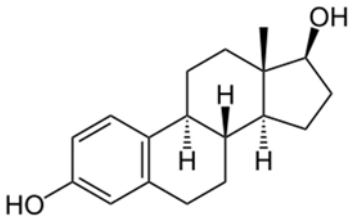
Introduction

Background

Estradiol ($C_{18}H_{24}O_2$), also known as E2, is a major female sex hormone (strongest estrogen) which regulates menstrual reproductive cycles as well as the estrous cycle. It is a key regulator of growth, differentiation, and function in a wide array of tissues, including the male and female reproductive tracts, mammary gland, brain, skeletal and cardiovascular systems.

E2 has two distinct intracellular receptors; ERα and ERβ which are mediating the main biological functions of E2. ERα and ERβ are encoded by unique genes possessing the functional domain characteristics of the steroid/thyroid hormone superfamily of nuclear receptors. ERα, as the predominant form, is expressed in the breast, uterus, cervix, and vagina., whereas ERβ exhibits a more limited pattern and is primarily expressed in the ovary, prostate, testis, spleen, lung, hypothalamus, and thymus.

Estradiol also influences bone growth, brain development and maturation, and food intake, and it is also critical in maintaining organ functions during severe trauma. In liver, Estradiol is conjugated to sulfate and glucuronide derivatives and excreted. Conversion to less-active estrogens, such as Estrone and Estriol (the major urinary metabolite) leads to deactivation of Estradiol.



Assay principle

The ZellX® Estradiol Immunoassay kit is a competitive ELISA assay designed to quantitatively measure Estradiol and its metabolites present in serum and plasma. **For analyzing Estradiol in urine, extracted dried fecal samples, and tissue culture media use our Estradiol ELISA assay Cat. No. ZX-55107-96.** An Estradiol 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 is pre-coated with a secondary donkey anti-sheep antibody. The function of this antibody is to capture the sheep anti-Estradiol antibody bound to Estradiol conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The Estradiol-conjugate (labeled) and the sample Estradiol (unlabeled) compete for binding to the sheep 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 Estradiol in the sample, the stronger the signal is, due to more labeled Estradiol bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Estradiol Standard (2400 pg/mL)	75 µL
Estradiol Antibody	2.6 mL
Estradiol 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 should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

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

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

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 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. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

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

The assay has been validated for the measurement of free Estradiol in both heparin plasma and serum samples. Serum and plasma samples must be diluted ≥ 1:20 with diluted Assay Buffer prior to performing the assay. Grossly lipemic or hemolyzed samples must be avoided. Estradiol is typically measured in serum, but Heparin plasma samples can also be utilized.

Normal adult human male serum Estradiol levels range between 10-40 pg/mL. Female levels are typically 15-350 pg/mL, decreasing to < 10 pg/mL after menopause. This assay may not be sensitive enough for all serum samples if the Estradiol level is < 2.05 pg/mL which is the threshold of the assay.

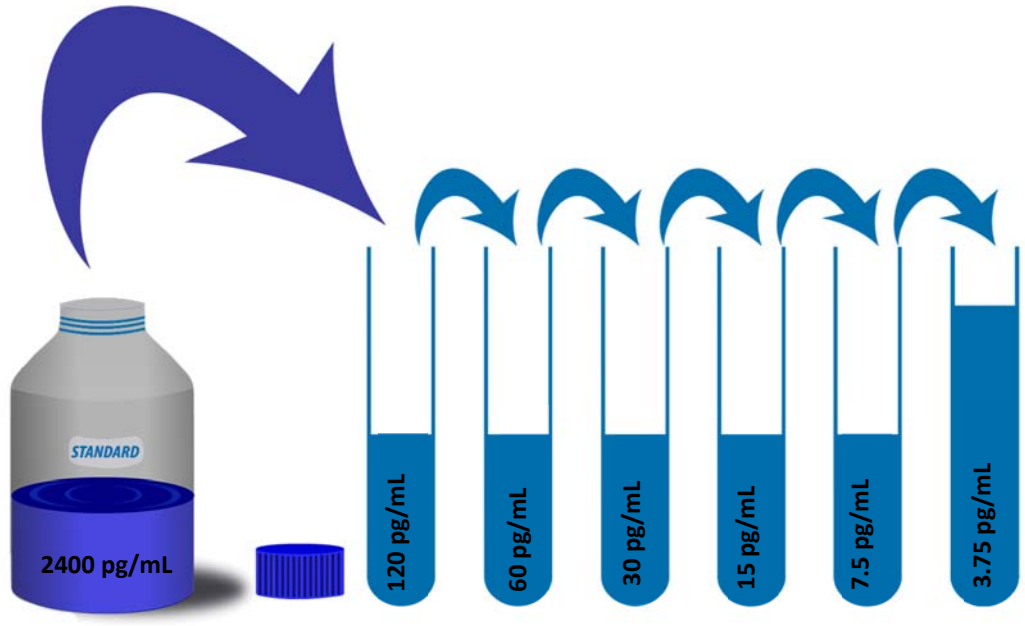
Please find further information about our steroid extraction protocols at https://zellx.de/protocol/ or contact us at technical@zellx.de

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

Standard preparation

- Prepare a 1:20 dilution of Estradiol Standard with Assay Buffer (mix 25 µL of standard with 475 µL of Assay Buffer), and label as the Standard No.6 (120 pg/mL).
- The Estradiol Standard contains an organic solvent. Prerinse the pipette tips 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.6	120 pg/mL	25 µL Estradiol Standard + 475 µL Assay Buffer
Standard No.5	60 pg/mL	250 µL Standard No.6 + 250 µL Assay Buffer
Standard No.4	30 pg/mL	250 µL Standard No.5 + 250 µL Assay Buffer
Standard No.3	15 pg/mL	250 µL Standard No.4 + 250 µL Assay Buffer
Standard No.2	7.5 pg/mL	250 µL Standard No.3 + 250 µL Assay Buffer
Standard No.1	3.75 pg/mL	250 µL Standard No.2 + 250 µL Assay Buffer
Standard No.0	0 pg/mL	250 µL Assay Buffer



All standard must be used within 2 hours of preparation

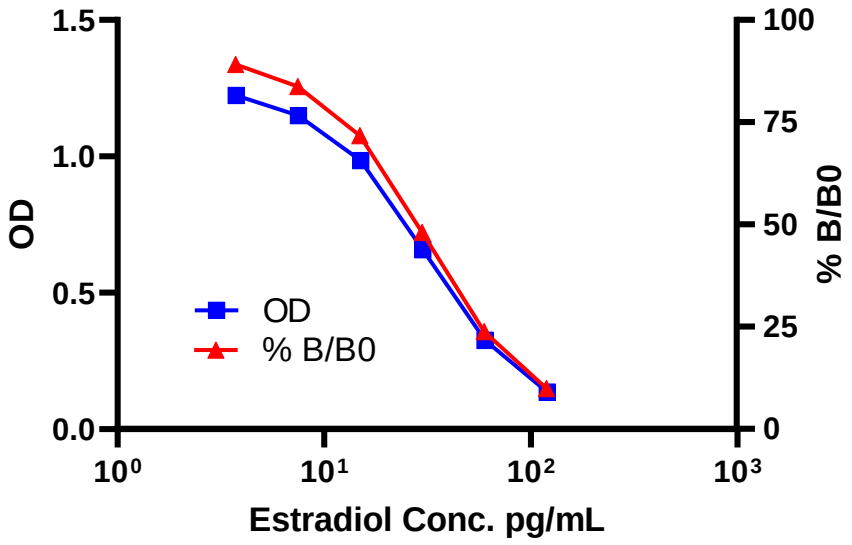
Assay Procedure

1. Pipette 100 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 100 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 125 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Estradiol Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Estradiol 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 20 % 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 obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 pg/mL of Estradiol is equivalent to 367.1 pM



A typical standard curve of ZellX® Estradiol ELISA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Estradiol ELISA assay was determined as 2.05 pg/mL.

Sensitivity

The sensitivity of the ZellX® Estradiol ELISA assay was determined as 2.21 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 17 different assays over multiple days.

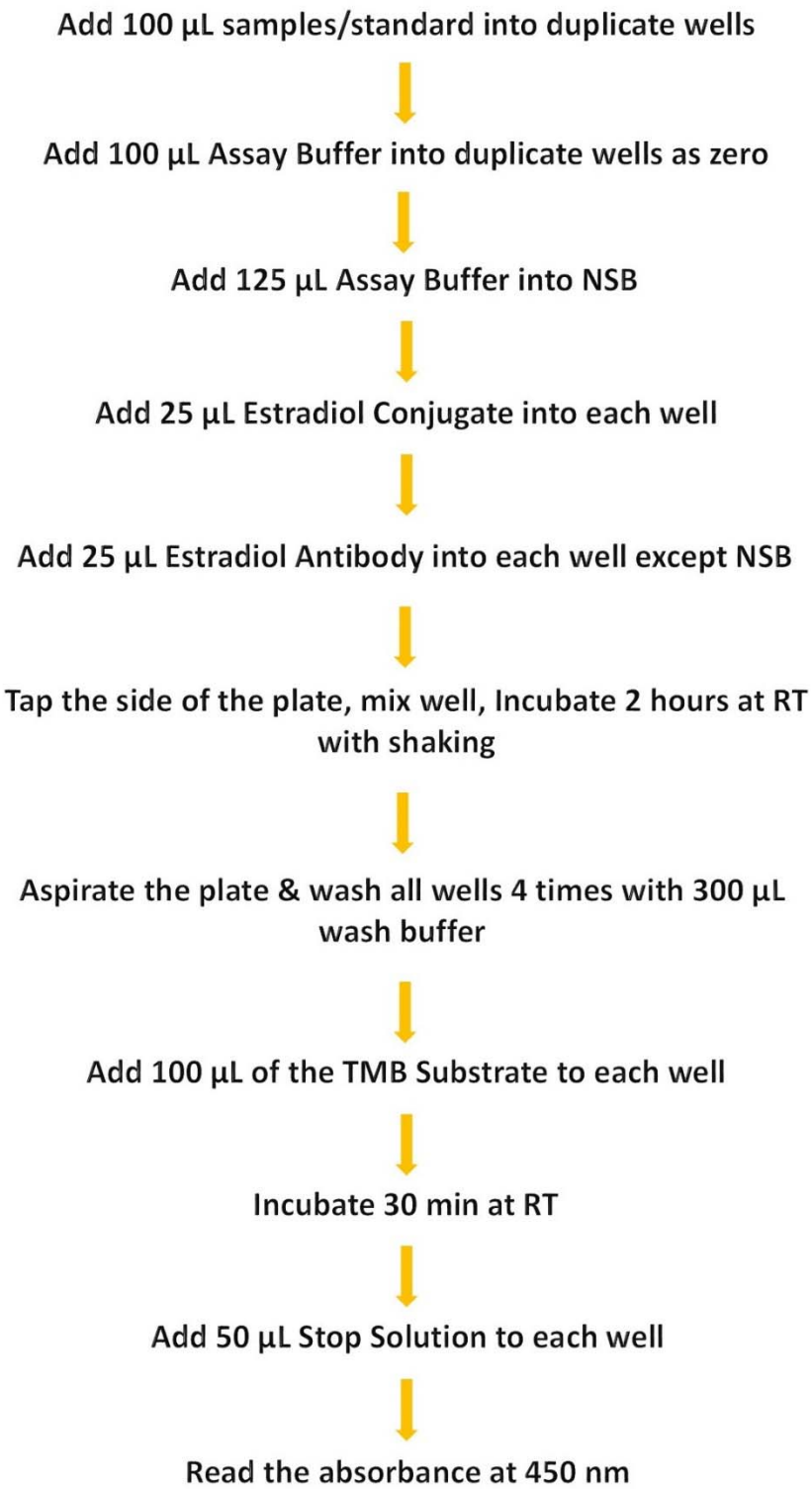
Item	*% CV*
Intra assay	4.1, 2.4, 5.1
Inter assay	9.8, 7.4, 9.6

Cross Reactivity

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

Steroid	*Cross Reactivity (%)*
17β -Estradiol	100
Estrone sulfate	3.20
Estrone	2.50
17α -Ethynylestradiol	< 0.3
Progesterone	< 0.3
Testosterone	< 0.3
5α-dihydroprogesterone	< 0.3
Cortisol	< 0.3
Corticosterone	< 0.3

Protocol summary



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