Estradiol

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55107-96

www.zellx.de

Sample Types Validated for:

Urine, 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.

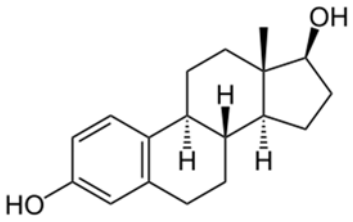
Introduction

Background

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 urine, extracted dried fecal samples, and tissue culture media. This kit is not recommended for serum, plasma, or saliva samples due to low concentration of Estradiol in such samples. **For analyzing Estradiol in serum and plasma use our High sensitivity Estradiol ELISA assay Cat. No. ZX-55108-96.** 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 goat anti-rabbit antibody. The function of this antibody is to capture the rabbit 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 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 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 (100 ng/mL)	125 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, and Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For Dried Fecal Sample:

ACS Grade Ethanol

Glass test tubes

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

This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to use. Estradiol can be assayed in other sample types, and for sample preparation method please contact us at technical@zellx.de.

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

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

I. **Urine:**

- Urine 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.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

II. **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.)**

III. **Tissue Culture Media:**

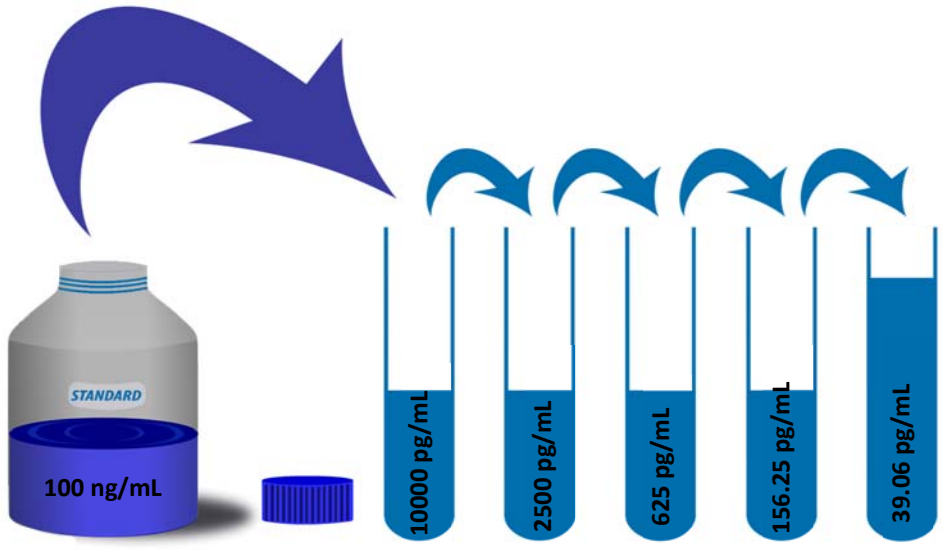
- For measuring Estradiol in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

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

Standard preparation

- Prepare a 1:10 dilution of Estradiol Standard with Assay Buffer (mix 50 µL of standard with 450 µL of Assay Buffer), and label as the Standard No.5 (10000 pg/mL).
- The Estradiol 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.5	10000 pg/mL	50 µL Estradiol Standard + 450 µL Assay Buffer
Standard No.4	2500 pg/mL	125 µL Standard No.5 + 375 µL Assay Buffer
Standard No.3	625 pg/mL	125 µL Standard No.4 + 375 µL Assay Buffer
Standard No.2	156.25 pg/mL	125 µL Standard No.3 + 375 µL Assay Buffer
Standard No.1	39.06 pg/mL	125 µL Standard No.2 + 375 µL Assay Buffer
Standard No.0	0 pg/mL	375 µL Assay Buffer



All standard must be used within 2 hours of preparation

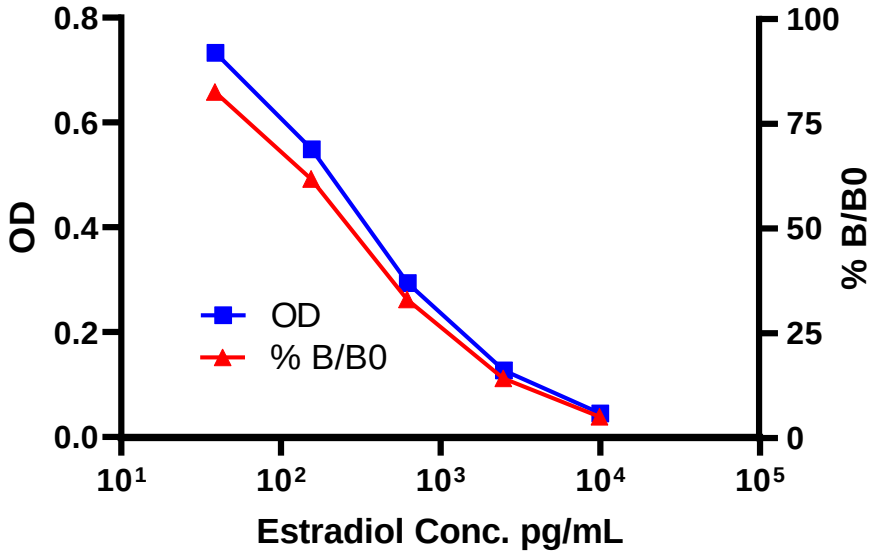
Assay Procedure

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 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 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 26.5 pg/mL.

Sensitivity

The sensitivity of the ZellX® Estradiol ELISA assay was determined as 39.6 pg/mL.

Precision

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

Inter-Assay Precision (Precision between assays): 3 human samples were tested in duplicate on 13 different assays over multiple days.

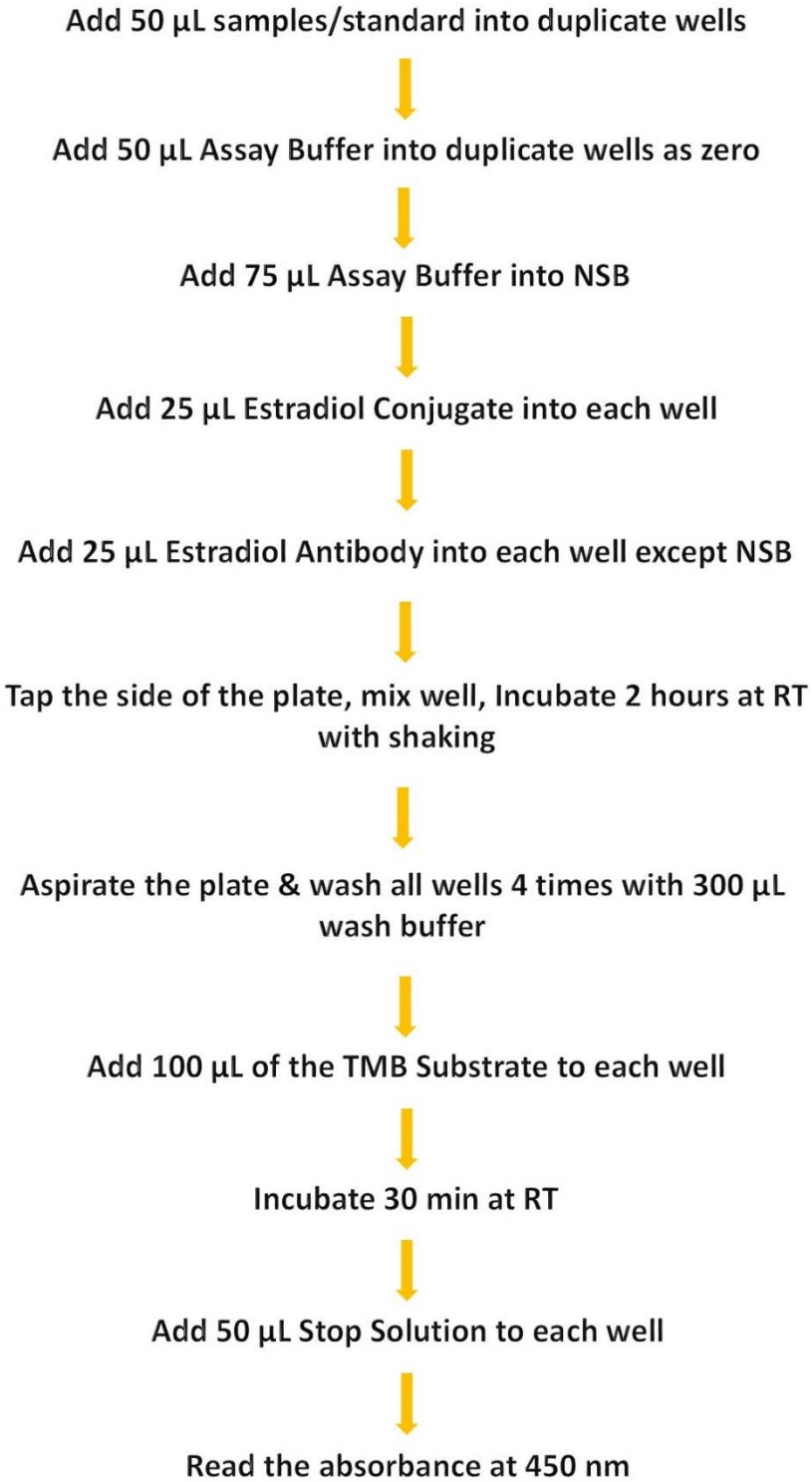
Item	*% CV*
Intra assay	4.0, 7.3, 3.9
Inter assay	13.8, 7.8, 3.6

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	0.78
17α -Estradiol	0.22
17α -Ethynylestradiol	0.11
Estrone Sulfate	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1
5α-dihydroprogesterone	< 0.1
Cortisol	< 0.1

Protocol summary



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