EliKine™ Estradiol ELISA Kit Booklet

Item NO.	Product Name
KET0003	EliKine™ Estradiol ELISA Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background

Estradiol (E2), also spelled oestradiol, is a steroid, an estrogen, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues such as the breasts, uterus, and vagina during puberty, adulthood, and pregnancy, but it also has important effects in many other tissues, including bone, fat, skin, liver, and the brain. While estrogen levels in men are lower compared to those in women, estrogens have essential functions in men, as well. It is found in most vertebrates and crustaceans, insects, fish, and other animal species.

Assay principle

EliKine™ Estradiol ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to estradiol. Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated estradiol. The competitive inhibition reaction is launched between HRP labeled estradiol and unlabeled estradiol with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of estradiol in the sample. The color development is stopped and the intensity of the color is measured.

Assay Restrictions

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

PRODUCT INFORMATION

Materials supplied

Estradiol microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with an antibody specific to estradiol.
Estradiol standard: Estradiol in a buffered protein base with preservatives, liquid.
HRP conjugated Estradiol: liquid.
HRP substrate A: Urea hydrogen peroxide solution.
HRP substrate B: TMB (Tetramethyl-benzidine) solution.
Stop solution: 2 mol/L sulfuric acid.
Wash buffer: PBS with 0.5% Tween-20, 20× liquid.
Plate covers

Other supplies required

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

Storage conditions

The unopened kit should be stored at 2-8 °C for 1 year. Immediately after use remaining reagents should be returned to cold storage at 4 °C. Recommended storage instruction for opened/reconstituted kit components are listed below.

Kit components	Storage conditions
Estradiol microplate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Estradiol standard	May be stored for up to 6 months at 2-8 °C.
HRP conjugated estradiol	May be stored for up to 1 year at 2-8 °C.
HRP substrate A	
HRP substrate B	
Stop solution	
Wash buffer (20×)	

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided.
3. Mix all reagents before using.
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

Technical hints

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

ASSAY PROTOCOL

Sample collection & storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.
Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Samples should be aliquoted and must be stored at -20 °C to avoid loss of bioactive estradiol. If samples are to be used within 24 hours, they may be stored at 2 to 8 °C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Reagent preparation

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.
Wash buffer - Dilute with Distilled or deionized water 1:20.
Sample dilution - If your samples need to be diluted, Deionized or distilled water is used for dilution of serum/plasma samples.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
2. Set a Blank well without any solution.
3. Add 50 µl of Standard or Sample per well. Standard need test in duplicate.
4. Add 50 µl of HRP-conjugate reagent to each well (not to Blank well). Mix well and then incubate for 1 hour at 37 °C.
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (250 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 50 µl of Substrate A and 50 µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37 °C. Keeping the plate away from drafts and other temperature fluctuations

in the dark.
7. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

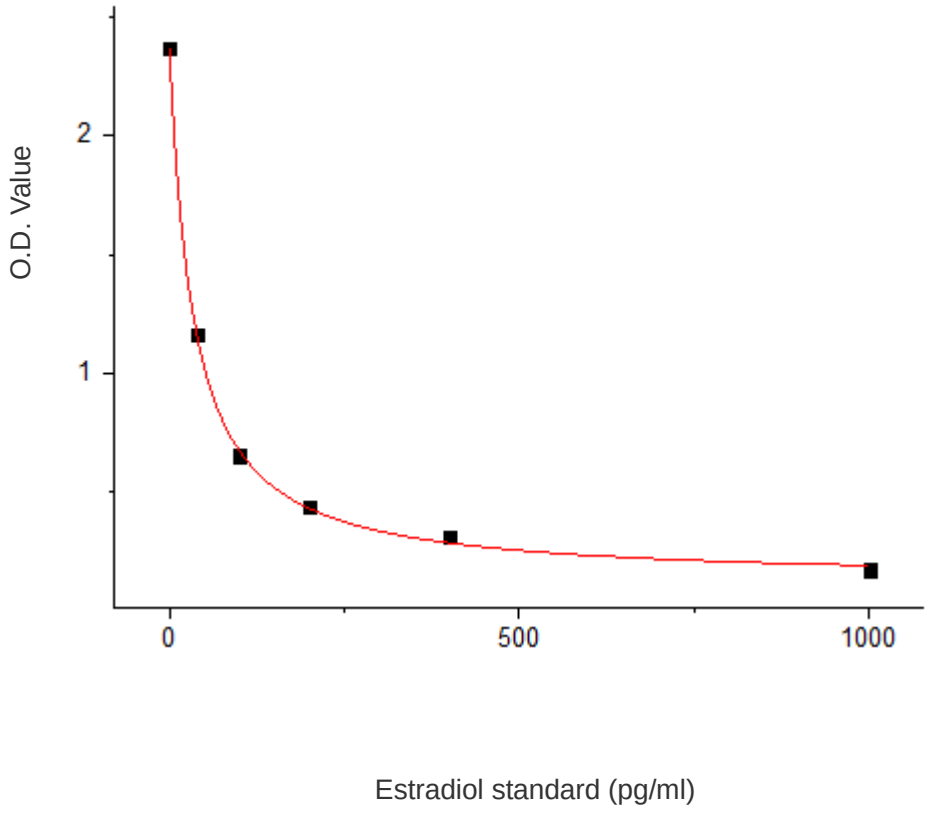
DATA ANALYSIS

Calculation of results

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.).
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the y-axis against the mean absorbance for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the estradiol concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical data

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.
Detection range: 40 pg/ml - 1000 pg/ml



Standard Estradiol (pg/ml)	Optical Density (450 nm)	Average
0	2.422	2.369
	2.316	
40	1.145	1.1615
	1.178	
100	0.639	0.655
	0.671	
200	0.450	0.441
	0.432	
400	0.317	0.3125
	0.308	
1000	0.168	0.1745
	0.181	

Precision

Intra-assay Precision (Precision within an assay)
Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜15%.
Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)＜15%.

Recovery

The recovery of estradiol spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.
The recovery ranged from 85% to 115% with an overall mean recovery of 100%.

Sensitivity

The minimum detectable dose (MDD) of estradiol is typically less than 40 pg/ml.
The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Linearity

To assess linearity of the assay, samples containing and/or spiked with high concentrations of estradiol were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Specificity

EliKine™ Estradiol ELISA Kit has high sensitivity and excellent specificity for detection of estradiol. No significant cross-reactivity or interference between estradiol and analogues was observed.

OTHER INFORMATION

Assay protocol summary

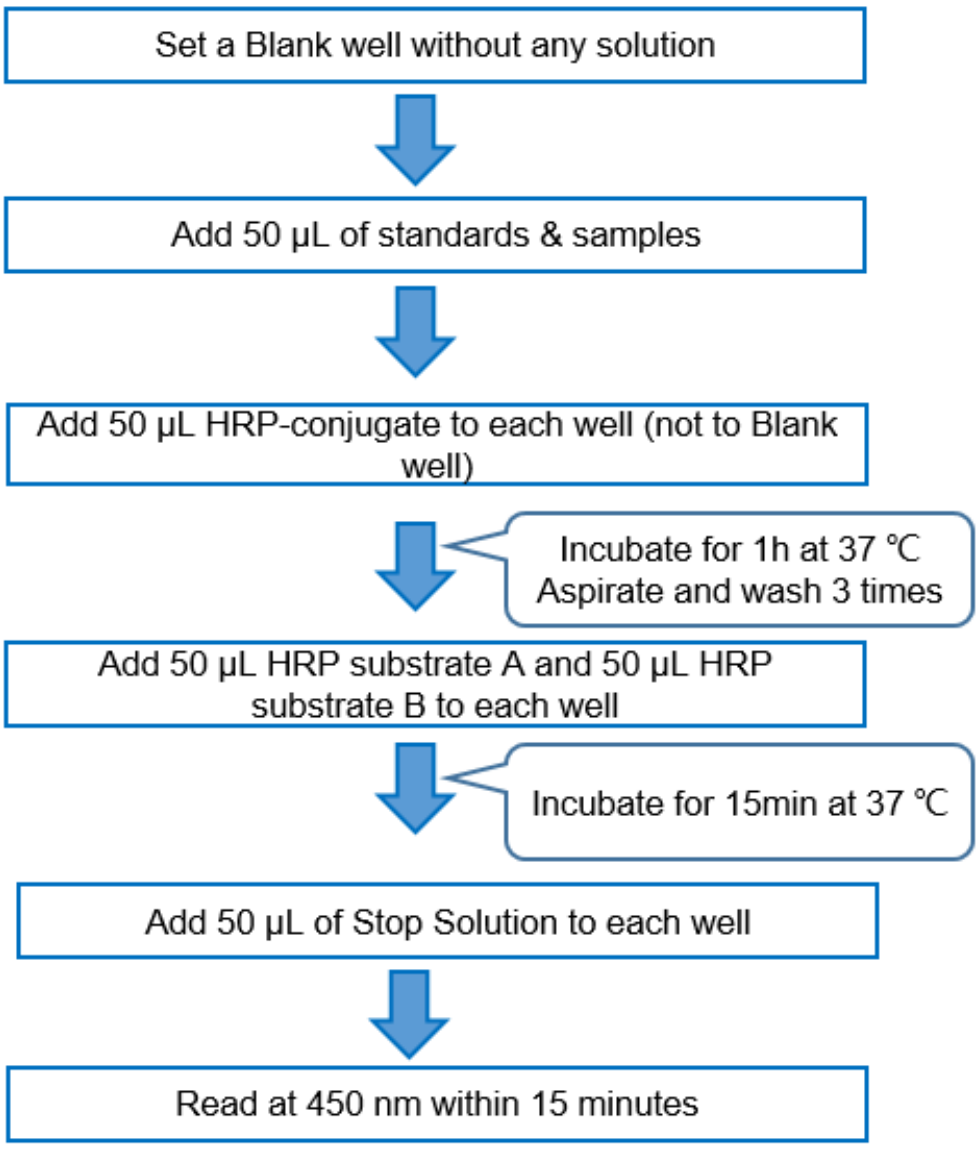


Plate layout