

Estradiol ELISA Kit

Catalog #: EIA-E2

User Manual

Last Revised: March 13, 2024

Introduction

The RayBio® Estradiol ELISA Kit is an in vitro quantitative assay for detecting Estradiol based on the competitive enzyme immunoassay (EIA) principle.

In this assay, the samples and standards are added to the plate, where endogenous Estradiol or the standards competes with the horseradish peroxidase (HRP) conjugated Estradiol for binding to the pre-coated anti-Estradiol antibody. After a wash step, any bound HRP conjugated Estradiol catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of bound HRP-Estradiol and inversely proportional to the amount of endogenous Estradiol in the standard or samples. A standard curve of known concentration of Estradiol can be established and the concentration of Estradiol in the samples can be calculated accordingly.

Storage / Stability

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store it at -80°C. Avoid repeated freeze-thaw cycles. For prepared reagent storage, see the kit components table on the next page.

Kit Components

Name	Catalog #	Size / Qty	Description	Storage / Stability After Preparation
<i>Anti-Estradiol Microplate</i>	EIA-E2-A	96 wells	Microplate coated with anti-Estradiol antibody.	1 month at 4°C*
<i>Estradiol Standard</i>	EIA-E2-STD	2 vials	Estradiol standard. 1 vial is enough to run each standard in duplicate.	1 month at 4°C
<i>HRP-Conjugated Estradiol</i>	EIA-E2-HRP	2 vials	HRP-conjugated Estradiol	First standard: 2-3 days at 4°C Other dilutions: N/A
<i>Estradiol Positive Control</i>	EIA-E2-PC	1 vial	Estradiol Positive Control	2-3 days at 4°C
<i>Wash Buffer</i>	EL-ITEMB	25 ml	20X concentrated wash buffer	1 month at 4°C
<i>Assay Diluent B</i>	EL-ITEME	15 ml	5X concentrated assay diluent	1 month at 4°C
<i>Sample Treatment Buffer 1</i>	EIA-STBI	11 ml	Serum/plasma sample treatment buffer 1	N/A
<i>Sample Treatment Buffer 2</i>	EIA-STBII	6 ml	Serum/plasma sample treatment buffer 2	N/A
<i>TMB One-Step Substrate Reagent</i>	EL-TMB	12 ml	3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution	N/A
<i>Stop Solution</i>	EL-STOP	8 ml	0.2 M sulfuric acid	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional Materials Required

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- SigmaPlot software (or similar 4-parameter logistic regression software)
- Tubes to prepare standard or sample dilutions
- Orbital shaker
- Distilled or deionized water
- Aluminum foil
- Plastic wrap

Sample Pre-Treatment

Note: For serum and plasma samples only. Sample pretreatment removes potentially interfering proteins and protein-bound Estradiol.

1. Add 200 μ l of sample and 50 μ l sample treatment buffer I to a microcentrifuge tube. The tubes will have a precipitate. Mix well.
2. Incubate for 15 minutes at room temperature.
3. Centrifuge at $\geq 12,000 \times g$ for 4 minutes.
4. Carefully remove and retain the supernatant. The supernatant may be cloudy until the addition of treatment buffer II.
5. Add 37.5 μ l of sample treatment buffer II to 125 μ l of the retained supernatant. Mix well.
6. The concentration read off the standard curve must be multiplied by the dilution factor, 1.6.

Reagent Preparation

Note: Keep kit reagents on ice during reagent preparation steps.

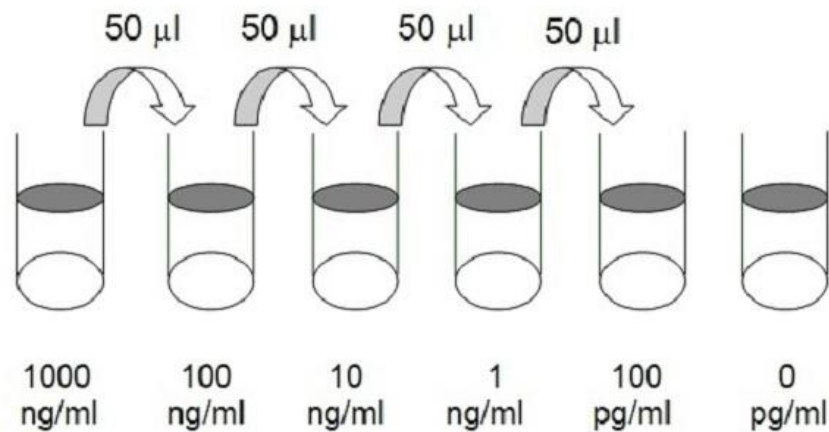
A. Preparation of Microplate and HRP-Conjugated Estradiol

1. Equilibrate plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the Estradiol-HRP vial. Then add 30 μ l of 1X Assay Diluent B to prepare the Estradiol-HRP concentrate. Pipette up and down to mix gently.
5. The Estradiol-HRP concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your Estradiol-HRP working solution, which will be used in step 2 of assay procedure.

B. Preparation of Standards

6. Label 5 microtubes with the following concentrations: 100 ng/ml, 10 ng/ml, 1 ng/ml, 100 pg/ml, and 0 pg/ml. Pipette 450 μ l of 1x Assay Diluent B into each tube.

- Briefly centrifuge the vial of Estradiol Standard. Add 495 μ l of 1x Assay Diluent B. Mix thoroughly. This solution serves as the first standard (1000 ng/ml).
- To make the 100 ng/ml standard, pipette 50 μ l of the 1000 ng/ml Estradiol standard into the tube labeled 100 ng/ml. Mix thoroughly.
- Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 μ l of 1x Assay Diluent B and 50 μ l of the prior concentration until the 0.1 ng/ml is reached. Mix each tube thoroughly before the next transfer.



C. Positive Control Preparation

- Briefly centrifuge the Positive Control vial. The Positive Control serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; if no positive competition is observed please contact RayBiotech Technical Support. The Positive Control may be diluted further if desired.

D. Sample Preparation

- If you wish to perform a dilution of your sample, dilute your sample with the 1x Assay Diluent B.

Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for pretreated serum:

Human=2X Mouse=2X Rat=2X

If you have any questions regarding the recommended dilutions, you may contact technical support at 770-729-2992 or techsupport@raybiotech.com.

E. Preparation of Wash Buffer

12. If 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved.
13. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 50 μ l of Estradiol-HRP (See Reagent Preparation step 5) to each well except blank wells. Add 50 μ l of each standard (see Reagent Preparation Section B), Positive Control (see Reagent Preparation Section C) and sample (see Reagent Preparation Section D) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C.
3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 μ l each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ l of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
5. Add 50 μ l of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 50 μ l Estradiol-HRP to each well.
3. Add 50 μ l standard or sample to appropriate well. Incubate 2.5 hours at room temperature or overnight at 4°C.
4. Add 100 μ l TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
5. Add 50 μ l Stop Solution to each well. Read at 450 nm immediately.

Calculations of Results

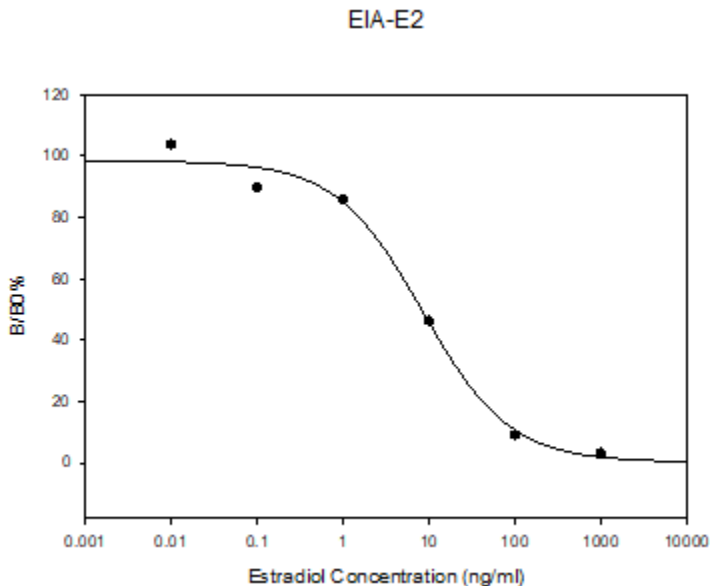
Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = $(B - \text{blank OD}) / (B_0 - \text{blank OD})$

B = OD of sample or standard

B₀ = OD of zero standard (total binding)

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of Estradiol is 1.5 ng/ml.

C. Standard Curve Range

0.1 - 1,000 ng/ml

D. Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<15%

E. Assay Diagram

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA10	SA10	SA18	SA18	SA26	SA26	SA34	SA34
Standard1	Standard1	SA3	SA3	SA11	SA11	SA19	SA19	SA27	SA27	SA35	SA35
Standard2	Standard2	SA4	SA4	SA12	SA12	SA20	SA20	SA28	SA28	SA36	SA36
Standard3	Standard3	SA5	SA5	SA13	SA13	SA21	SA21	SA29	SA29	SA37	SA37
Standard4	Standard4	SA6	SA6	SA14	SA14	SA22	SA22	SA30	SA30	SA38	SA38
Standard5	Standard5	SA7	SA7	SA15	SA15	SA23	SA23	SA31	SA31	SA39	SA39
Pos Control	Pos Control	SA8	SA8	SA16	SA16	SA24	SA24	SA32	SA32	SA40	SA40

Key:

Blank = Buffer Only

Total Binding = Estradiol only

Standard 1 = 1000 ng/ml

Standard 2 = 100 ng/ml

Standard 3 = 10 ng/ml

Standard 4 = 1 ng/ml

Standard 5 = 0.1 ng/ml

Pos Control = Positive Control

F. Specificity

This EIA kit is designed to detect human, mouse, and rat Estradiol.

Troubleshooting Guide

<i>Problem</i>	<i>Cause</i>	<i>Solution</i>
<i>Poor standard curve</i>	<ul style="list-style-type: none"> Inaccurate pipetting Improper standard dilution 	<ul style="list-style-type: none"> Check pipettes Briefly centrifuge standard and dissolve the powder thoroughly by gently mixing
<i>Low signal</i>	<ul style="list-style-type: none"> Improper preparation of standard and/or biotinylated antibody Too brief incubation times Inadequate reagent volumes or improper dilution 	<ul style="list-style-type: none"> Briefly spin down vials before opening. Dissolve the powder thoroughly. Ensure sufficient incubation time; assay procedure step 2 may be done overnight Check pipettes and ensure correct preparation
<i>Large CV</i>	<ul style="list-style-type: none"> Inaccurate pipetting Air bubbles in wells 	<ul style="list-style-type: none"> Check pipettes Remove bubbles in wells
<i>High background</i>	<ul style="list-style-type: none"> Plate is insufficiently washed Contaminated wash buffer 	<ul style="list-style-type: none"> Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed Make fresh wash buffer
<i>Low sensitivity</i>	<ul style="list-style-type: none"> Improper storage of the ELISA kit Stop solution 	<ul style="list-style-type: none"> Follow storage recommendations. Keep substrate solution protected from light. Add stop solution to each well before reading plate