3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

Progesterone ELISA Kit

Catalog #: EIA-P4

User Manual
Last Revised: March 15, 2024

Introduction

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

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

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

Kit Components

Name	Catalog #	Size / Qty	Description	Storage / Stability After Preparation
Progesterone Microplate	EIA-P4-A	96 wells	Microplate coated with Progesterone	1 month at 4°C*
Progesterone Standard	EIA-P4-STD	2 vials	Progesterone standard. 1 vial is enough to run each standard in duplicate.	1 month at 4°C
HRP-Conjugated Progesterone	EIA-P4-HRP	2 vials	HRP-conjugated Progesterone	First standard: 2-3 days at 4°C Other dilutions: N/A
Progesterone Positive Control	EIA-PC	1 vial	Progesterone Positive Control	2-3 days at 4°C
Wash Buffer	EL-ITEMB	25 ml	20X concentrated wash buffer	1 month at 4°C
Assay Diluent B	EL-ITEME	15 ml	5X concentrated assay diluent	1 month at 4°C
TMB One-Step Substrate Reagent	EL-TMB	12 ml	3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	N/A
Stop Solution	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

Reagent Preparation

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

RayBiotech
Empowering your proteomics

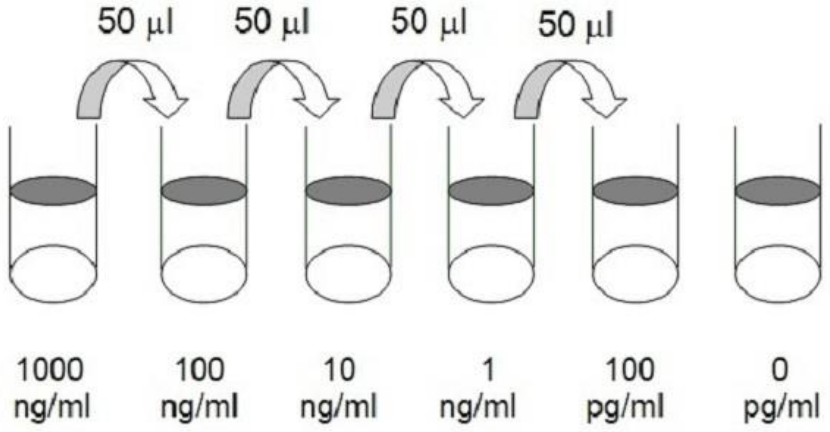
3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

A. Preparation of Microplate and HRP-Conjugated Progesterone

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 Progesterone-HRP vial. Then add 30 µl of 1X Assay Diluent B to prepare the Progesterone-HRP concentrate. Pipette up and down to mix gently.
5. The Progesterone-HRP concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your Progesterone-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 µl of 1x Assay Diluent B into each tube.
7. Briefly centrifuge the vial of Progesterone Standard. Reconstitute with 500ul µl of 1x Assay Diluent B. Mix thoroughly. This solution serves as the first standard (1000 ng/ml).
8. To make the 100 ng/ml standard, pipette 50 µl of the 1000 ng/ml Progesterone standard into the tube labeled 100 ng/ml. Mix thoroughly.
9. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µl of 1x Assay Diluent B and 50 µl of the prior concentration until the 0.1 ng/ml is reached. Mix each tube thoroughly before the next transfer.



3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

C. Positive Control Preparation

10. Briefly centrifuge the Positive Control vial (Item M) and reconstitute with 100 µl of ddH2O. 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

11. 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=8X Mouse=8X Rat=8X

    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 µl of Progesterone-HRP (See Reagent Preparation step 5) to each well except blank wells. Add 50 µl 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 4ºC.

3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer.

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

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 µl 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 µl 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 µl Progesterone-HRP to each well.
3. Add 50 µl standard or sample to appropriate well. Incubate 2.5 hours at room temperature or overnight at 4ºC.
4. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
5. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

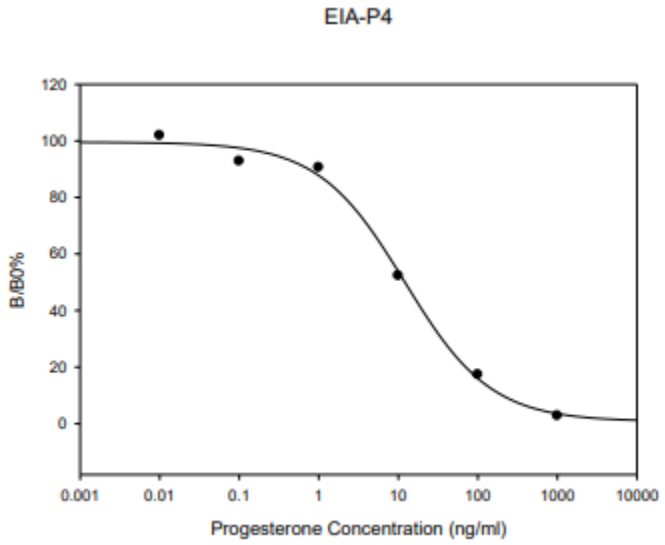
Calculations of Results

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-blank OD)/(B0-blank OD)
B = OD of sample or standard
B0 = OD of zero standard (total binding)

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

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 Progesterone is 2.1 ng/ml.

C. Standard Curve Range

0.1 - 1,000 ng/ml

D. Reproducibility

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

RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

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 = Progesterone 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 Progesterone.

RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

Troubleshooting Guide

Problem	*Cause*	*Solution*
Poor standard curve	• Inaccurate pipetting Improper standard dilution	• Check pipettes • Briefly centrifuge standard and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times Inadequate reagent volumes or improper dilution	• 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
Large CV	• Inaccurate pipetting Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Follow storage recommendations. Keep substrate solution protected from light. • Add stop solution to each well before reading plate