# RayBio® Human/Mouse/Rat Cortisol Enzyme Immunoassay Kit

Catalog #: EIA-CORT, EIAM-CORT, EIAR-CORT

User Manual
Last revised Feb. 15th, 2021

Caution:
Extraordinarily useful information enclosed


ISO 13485 Certified

3607 Parkway Lane, Suite 100
Norcross, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax:770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

# Table of Contents



Please read the entire manual carefully before starting your experiment

# I. Introduction

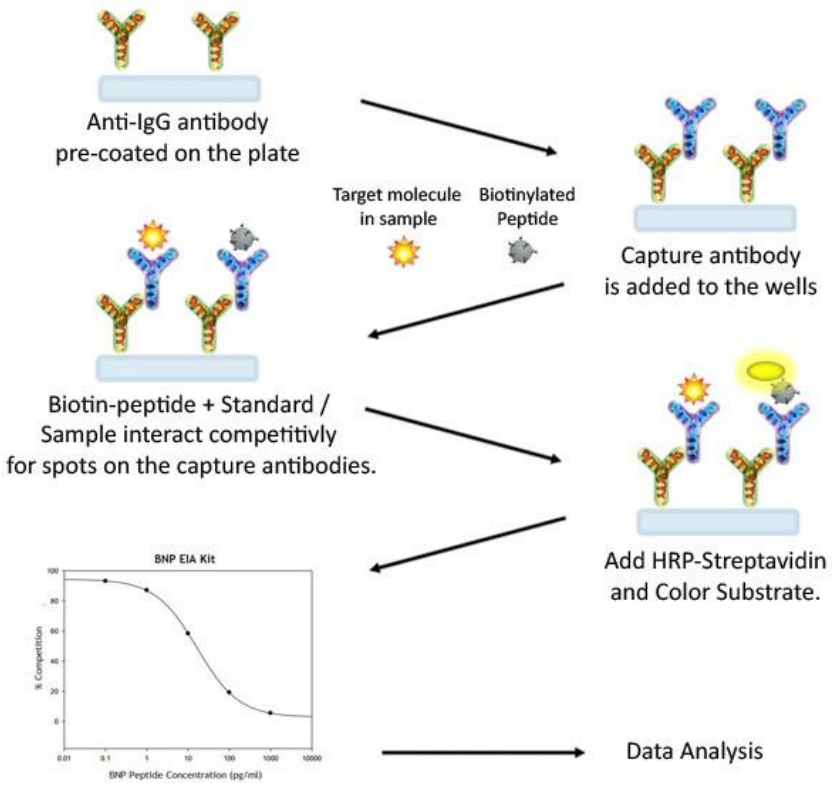
Cortisol is a naturally occurring pregnane corticosteroid and is also known as 11β,17α,21-trihydroxypregn-4-ene-3,20-dione.  Cortisol is synthesized from cholesterol mainly by the adrenal gland in the zona fasciculata. Release of Cortisol is increased in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid in the metabolism of fat, protein, and carbohydrates. It also decreases bone formation.

# II. General Description

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

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

# III. How It Works



# IV. Storage

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 at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see table below.

# V. Reagents

| **Component** | **Size / Description** | **Storage / Stability <u>After Preparation</u>** |
|---|---|---|
| EIA Microplate (Item A) | 96 wells (12 strips x 8 wells) coated with secondary antibody. | 1 month at 4°C* |
| Wash Buffer Concentrate (20X) (Item B) | 25 ml of 20X concentrated solution. | 1 month at 4°C |
| Standard Cortisol (Item C) | 2 vials of Cortisol. 1 vial is enough to run each standard in duplicate. | The first standard: 2-3 days at 4°C Additional dilutions: Do not store |
| Anti-Cortisol Polyclonal Antibody (Item N) | 2 vials of anti-Cortisol. | 1 month at 4°C |
| Assay Diluent B (Item E) | 15 ml of 5X concentrated buffer. Diluent for standards, cell culture media or other sample types, and HRP-Conjugate. | 1 month at 4°C |
| HRP Conjugated Cortisol (Item F) | 2 vials of HRP Conjugated Cortisol, 1 vial is enough to assay the whole plate. | 2-3 days at 4°C |
| Positive Control (Item M) | 1 vial of Positive Control. | 2-3 days at 4°C |
| TMB One-Step Substrate Reagent (Item H) | 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution. | N/A |
| Stop Solution (Item I) | 8 ml of 0.2 M sulfuric acid. | N/A |

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

# VI. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil
11. Plastic wrap

## VII. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

### A. Preparation of Plate and Anti-Cortisol Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.

2. Label removable 8-well strips as appropriate for your experiment.

3. 5X Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

4. Briefly centrifuge the anti-Cortisol antibody vial (Item N). Then add 50 µl of 1X Assay Diluent B to the vial to prepare the antibody concentrate. Pipette up and down to mix gently.

5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent **B.** This is your anti-Cortisol antibody working solution, which will be used in step 2 of Assay Procedure (Section VIII).

*Note: The following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure)*

## B. Preparation of HRP Conjugated Cortisol (Item F)

6. Briefly centrifuge the vial of HRP Conjugated Cortisol (Item F) before use.

7. Add 30 µl of 1X Assay Diluent B to prepare the Cortisol-HRP concentrate. Pipette up and down to mix gently.

8. The Cortisol-HRP concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your Cortisol-HRP working solution, which will be used in step 4 of Assay Procedure (Section VIII).

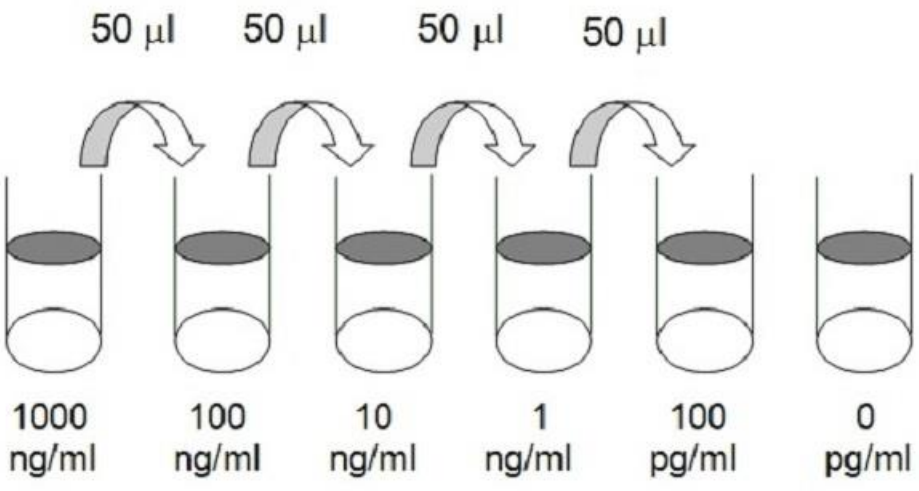
## C. Preparation of Standards

8. 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.

9. Briefly centrifuge the vial of Cortisol Standard (Item C). Add 495 µl of 1x Assay Diluent B. Mix thoroughly. This solution serves as the first standard (1000 ng/ml).

10. To make the 100 ng/ml standard, pipette 50 µl of the 1,000 ng/ml Cortisol standard into the tube labeled 100 ng/ml. Mix thoroughly.

11. 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 100 pg/ml is reached. Mix each tube thoroughly before the next transfer.



## D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial (Item M). Briefly centrifuge the Positive Control vial (Item M). 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.

## E. Sample Preparation

13. 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 serum:
    Human=8X Mouse=2X Rat=2X.
    If you have any questions regarding the recommended dilutions you may contact technical support at 888-494-8555 or techsupport@raybiotech.com.

**F. Preparation of Wash Buffer**

14. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved.

15. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

# VIII. 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 100 µl of Anti-Cortisol Antibody (Item N) (See Reagent Preparation step 5) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycle/sec). You may also incubate 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. 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 50 µl of Cortisol-HRP (Item F) (See Reagent Preparation Section B) to each well except blank wells. Add 50 µl of each standard (see Reagent Preparation Section C), Positive Control (see Reagent Preparation Section D) and sample (see Reagent Preparation Section E) 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.

5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

## IX. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl anti-Cortisol to each well. Incubate 1.5 hours at room temperature or overnight at 4ºC.

3. Add 50 µl standard or sample to each 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.

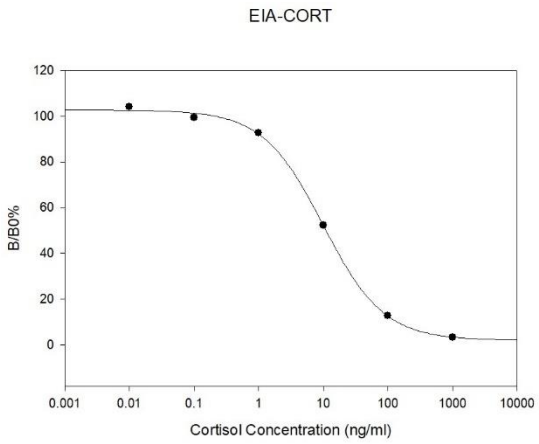
# X. Calculation of Results

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)/($B_0$-blank OD) where
B = OD of sample or standard and
$B_0$ = 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 concentrations of Cortisol is 2.7 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

Recommended Plate Layout:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 = Biotin-Cortisol only Standard

1 = 1,000 ng/ml

Standard 2 = 100 ng/ml

Standard 3 = 10 ng/ml

Standard 4 = 1 ng/ml

Standard 5 = 100 pg/ml

Pos Control = Biotin with Item M

# XI. Specificity

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

# XIV. Publications Citing This Product

1. KyrÃ¶nlahti A, et al. GATA4 regulates Sertoli cell function and fertility in adult male mice. Molecular and Cellular Endocrinology Volume 333, Issue 1, 10 February 2011, Pages 85Â–95
   **Species: Mouse**
   **Sample Type:**
2. Satie AP., et al. Excess Type I Interferon Signaling in the Mouse Seminiferous Tubules Leads to Germ Cell Loss and Sterility. J Biol Chem. 2011 Jul 1;286(26):23280-95. doi: 10.1074/jbc.M111.229120
   **Species: Mouse**
   **Sample Type:**

# XIII. Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | ○ Inaccurate pipetting<br>○ Improper standard dilution | ○ Check pipettes<br>○ Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing |
| Low signal | ○ Improper preparation of standard and/or HRP Conjugate<br>○ Too brief incubation times<br>○ Inadequate reagent volumes or improper dilution | ○ Briefly spin down vials before opening. Dissolve the powder thoroughly.<br>○ Ensure sufficient incubation time; assay procedure step 2 may be done overnight<br>○ Check pipettes and ensure correct preparation |
| Large CV | ○ Inaccurate pipetting<br>○ Air bubbles in wells<br>○ | ○ Check pipettes<br>○ Remove bubbles in wells<br>○ |
| High background | ○ Plate is insufficiently washed<br>○ Contaminated wash buffer<br>○ | ○ Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.<br>○ Make fresh wash buffer<br>○ |
| Low sensitivity | ○ Improper storage of the ELISA kit<br>○ Stop solution<br>○ | ○ Follow storage recomendations in sections IV and V. Keep substrate solution protected from light.<br>○ Add stop solution to each well before reading plate |

# RayBio® ELISA Kits

Over 3,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.