KAMIYA BIOMEDICAL COMPANY

# Mouse and Rat Corticosterone ELISA

**For the quantitative determination of corticosterone in mouse and rat biological fluids**

Cat. No. KT-510

**For Research Use Only.**

## PRODUCT INFORMATION

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## INTENDED USE

The Mouse and Rat Corticosterone ELISA is for the quantitative determination of corticosterone in mouse and rat biological fluids. For research use only.

## INTRODUCTION

Corticosterone ($C_{21}H_{30}O_4$, also called 11ß,21-Dihydroxyprogesterone, Reichstein's Substance H, or Kendall's Compound B) is, like cortisol and cortisone, a glucocorticoid hormone secreted from the cortex of the adrenal gland. Corticosterone is derived from cholesterol through a series of enzymatically mediated steps and also serves as a precursor of aldosterone. It is a primary glucocorticoid in mice, rats and other animals (such as rabbits, birds, amphibians, and reptilians) in which the 17α-hydroxylase is supposed to not exist in the adrenal gland. Corticosterone is produced under the control of ACTH and the production has a circadian rhythm with peak levels in the latter portion of the day in nocturnal animals like rats and is believed to play a decisive role in sleep-wake cycles. Corticosterone can be used as a non-invasive biomarker of stress study through the collection of urine and feces to avoid corticosterone increase in blood levels which is caused by normal invasive methods. Corticosterone is also being studied in different fields such as impairment of long-term memory retrieval, chronic corticosterone elevation due to dietary restrictions and response to burn injuries etc.

Since most of corticosterone in blood is bound to a plasma protein called corticosterone-binding globulin (CBG), the determination of blood corticosterone with presently available commercial assay kits requires an initial extraction procedure. On the other hand, the present assay kit for corticosterone newly developed by our laboratory provides a tool for direct determination of corticosterone in blood by simple dilution of blood samples with the diluent included in the kit. Furthermore, assays using the kit can be completed within a short period. The corticosterone ELISA kit newly developed will be quite a useful tool for further development of corticosterone research.

## PRINCIPLE

This ELISA kit is used for the quantitative determination of corticosterone in biological fluids such as plasma, serum or urine samples of mice, rats and other species, and also cell or tissue culture supernatant. It has various advantages, such as no extraction procedure of samples, short assay time, and practically no influences of other body fluids or physiological active substances coexisting in samples assayed.

This ELISA kit for the determination of corticosterone is based on a competitive enzyme immunoassay using a combination of specific antibodies to corticosterone and corticosterone-HRP conjugate (HRP-labeled corticosterone) system. The 96 well plate is coated with goat anti rabbit IgG, to which corticosterone calibrator or samples, HRP-labeled corticosterone and specific antibody are added for competitive immunoreaction. After incubation and plate washing, HRP enzyme activity is determined by 3,3',5,5'-tetramethylbenzidine (TMB) and the concentration of corticosterone is calculated.

## COMPONENTS

| Component | Form | Quantity | Main Ingredient |
|---|---|---|---|
| 1. Antibody-Coated Plate | Microtiter plate | 1 plate (96-well) | Goat anti-rabbit IgG |
| 2. Corticosterone Calibrator | Lyophilized | 1 vial (50 ng) | Synthetic corticosterone |
| 3. HRP-Labeled Corticosterone | Liquid | 1 vial (0.3 mL) | HRP conjugated corticosterone |
| 4. Specific Antibody | Liquid | 1 bottle (7 mL) | Rabbit anti-corticosterone antibody |
| 5. TMB Substrate | Liquid | 1 bottle (12 mL) | TMB (3,3',5,5'-tetramethylbenzidine) |
| 6. Stop Solution | Liquid | 1 bottle (12 mL) | 1M $H_2SO_4$ |
| 7. Buffer Solution | Liquid | 1 bottle (10 mL) | PBS buffer containing protein stabilizer |
| 8. Sample Diluent | Liquid | 1 bottle ( 50 mL) | A specially formulated displacer of CBG |
| 9. Wash Solution Concentrate | Liquid | 1 bottle (25 mL) | Concentrated saline |
| 10. Adhesive Foil | | 2 sheets | |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
- Washing device for microtiter plate and dispenser with aspiration system (optional)
- Micropipettes for volumes between 10 µL – 1,000 µL
- Multi-channel pipettes for 8 wells or 12 wells and their tips
- Glass test tubes for preparation of calibrator and sample solutions
- Graduated cylinder (500 mL or 1,000 mL)
- Distilled or de-ionized water
- A microplate shaker (210-240 rpm)

## NOTES

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Color reaction should be carried out in the dark.

Read optical absorbance of the reaction solution in wells as soon as possible after stopping the color reaction.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette corticosterone calibrator solutions or samples into the wells of the assay plate. In addition, use clean test tubes or vessels in the assay and a new tip for each calibrator diluting process and for each sample or calibrator solution pipetting to avoid cross-contamination.

To quantitate accurately, run a calibration curve for each assay.

Corticosterone calibrator solutions and HRP-labeled corticosterone solution should be prepared immediately before use. If the kit is to be divided, the rest of the reconstituted corticosterone calibrator solution (50 ng/mL), HRP-labeled corticosterone solution and other reagents except wash solution and stop solution should be stored at 4℃ and used within 2 weeks. Diluted calibrator solutions, except the 50 ng/mL calibrator, should not be reused for another assay.

It is recommended that serum or plasma samples should be used as soon as possible after collection. If the sample is to be tested later, it should be aliquoted and frozen below -30℃ (for long term storage, in a -80℃ freezer). Avoid repeated freezing and thawing of samples.

The recommended diluting ratio for mouse or rat samples is 20 - 100 fold, but corticosterone levels significantly differ among animals and also show marked circadian variation even in the same individual. Therefore, optimal dilution tests should be run when handling test samples of other species other than mice or rats. In addition, since progesterone levels increase significantly in pregnant animals, the cross reactivity of the steroid should be considered when such samples are tested.

Incomplete washing of the microplate will interfere with assay precision. If a microplate washer is not available, completely aspirate the solution in the wells of the assay plate to be removed or decant them by inverting the plate and tapping it onto absorbent tissue in each wash cycle. Ensure that there is no residual wash solution in the wells after the final wash.

## REAGENT PREPARATION

1. Preparation of Calibrator Solution: Reconstitute the lyophilized Corticosterone Calibrator (50 ng/vial) with 1 mL of Sample Diluent, which affords a 50 ng/mL Calibrator Solution. Dilute 0.2 mL of the 50 ng/mL Calibrator Solution with 0.4 mL of Sample Diluent, which yields a 16.67 ng/mL Calibrator Solution. Repeat the same dilution procedure to make 5.56, 1.85, 0.62, and 0.21 ng/mL Calibrator Solutions. Sample Diluent is used as the 0 ng/mL Calibrator.

2. Preparation of HRP-labeled corticosterone solution: Take 0.25 mL of HRP-labeled Corticosterone from the labeled vial and dilute with 7 mL of Buffer Solution.

3. Dilution of Wash Solution Concentrate: Dilute one bottle of Wash Solution Concentrate (25 mL) to 500 mL with distilled or de-ionized water.

4. The other reagents are ready for use.

## ASSAY SAMPLE PREPARATION

1. For mouse/rat plasma and serum: Dilute 10 µL of plasma or serum sample with 400 µL of Sample Diluent in a test tube. Mix the diluted solution and allow it to stand for 10 minutes at room temperature.

2. For mouse/rat urine: Dilute 10 µL of urine sample (40 to 100 fold) with Sample Diluent in a test tube. Mix the diluted solution and allow it to stand for 10 minutes at room temperature.

3. For culture supernatant (RPMI1640 with or without FCS): Dilute 50 µL of supernatant with 250 µL of Sample Diluent in a test tube. Mix the diluted solution and allow it to stand for 10 minutes at room temperature.

4. For other species or matrix samples: Because corticosterone concentrations are different significantly in various species of animals, it is recommended that a series of diluted samples are prepared and tested to find the optimal dilution ratio before assay.

## STORAGE

Store kit at 4°C. The kit is stable until expiration date listed on box.

## ASSAY PROTOCOL

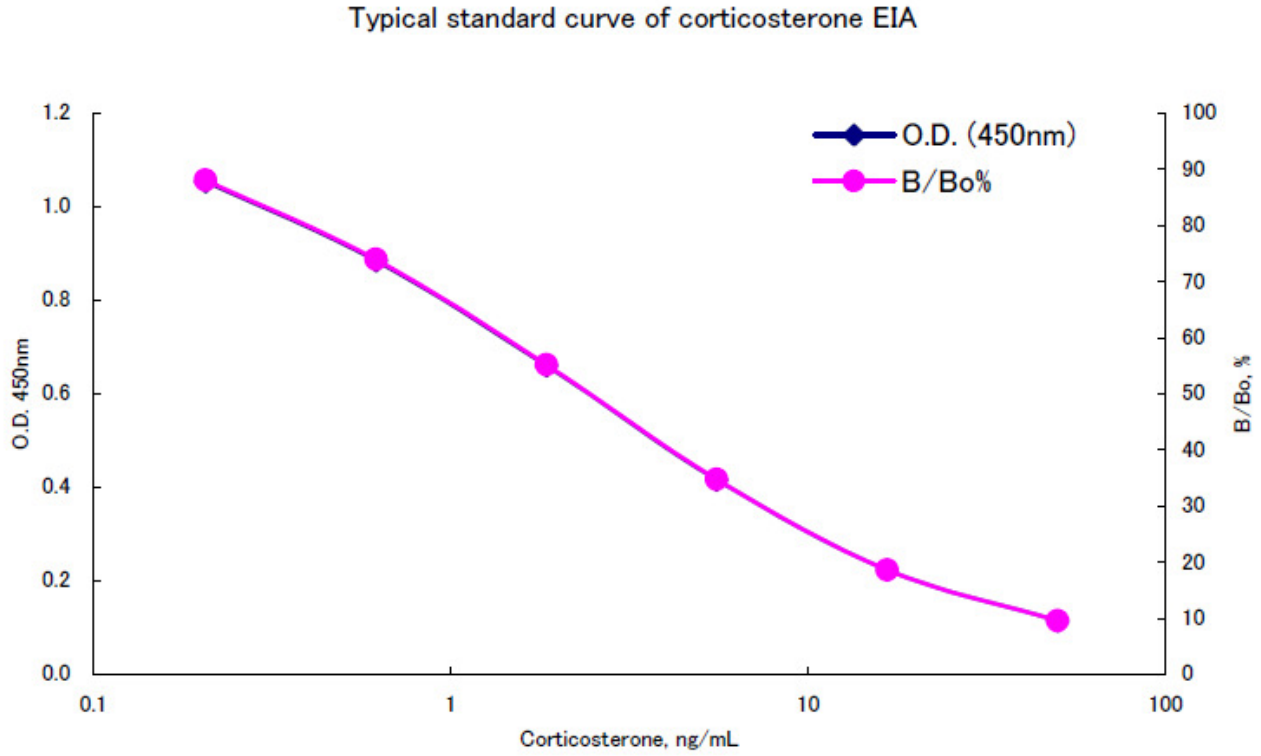
1. Bring all the reagents, except test samples, to room temperature (22-25℃) before starting assay.
2. Add 350 µL of diluted Wash Solution to each well and keep it for about 30 seconds, and then aspirate or decant the wash solution in the wells. Invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure blotting free of most of the residual Wash Solution.
3. Pipette 100 µL of corticosterone calibrator solutions (0, 0.21, 0.62, 1.85, 5.56, 16.67, and 50 ng/mL) or diluted samples, after being vortexed, into appropriate wells. Add 50 µL of HRP-labeled corticosterone solution into each well, and finally add 50 µL of Specific Antibody into each well.
4. Cover the plate with adhesive foil and incubate it on a shaker at 210-220 rpm room temperature for two hours.
5. After incubation, take off the adhesive foil, aspirate or decant the solutions in the wells. Add 350 µL of diluted wash solution to each well and keep it for about 30 seconds, and then aspirate or decant the wash solution in the wells. Repeat this wash process 4 times (total 5 times). Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure blotting free of most of the residual Wash Solution.
6. Add 100 µL of TMB Substrate into each well.
7. Cover the plate with adhesive foil and incubate it on a shaker at 210-220 rpm at room temperature for 30 minutes.
8. Add 100 µL of Stop Solution into each of the wells to stop color reaction.
9. Read optical absorbance of the solution in the wells at 450 nm.

## RESULTS

The assay fits best to a 4-parameter logistic equation, Y= (a-d)/(1+(x/c)^b)+d; here a,b,c,d represent constant parameter. Alternatively, calculate mean optical density values of wells containing calibrators or their percent bound to maximum binding wells (0 ng/mL) and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of calibrator; ordinate: optical density values or bound%). Use the average optical density or bound% of each sample to determine the corresponding value by simple interpolation from this calibration curve. The results should be multiplied by the dilution factor to obtain the actual concentrations for undiluted unknown samples. Perform all the determinations in duplicate.

## PERFORMANCE

**Typical Calibration Curve** (example only, a new calibration curve for each run must be established by the end-user)




**Assay Range**

0.21 – 50 ng/mL

**Sensitivity**

Sensitivity can be calculated using the following formula under the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols.

$$\text{Sensitivity (ng/mL)} = \frac{\text{2 X SD of the Zero Calibrator X 0.21 ng/mL}}{\text{(Optical Density of 0 ng/mL – Optical density of 0.21 ng/mL)}}$$

**Precision and Reproducibility**

| | Intra-assay variation (mean±SD, n=10) | | Inter-assay variation (mean±SD, n=9) | |
|---|---|---|---|---|
| | Measured (ng/mL) | %CV | Measured (ng/mL) | %CV |
| QC sample 1 | 0.767±0.036 | 4.7 | 0.767±0.063 | 8.2 |
| QC sample 2 | 2.802±0.105 | 3.7 | 2.655±0.205 | 7.7 |
| QC sample 3 | 7.837±0.197 | 2.5 | 6.951±0.683 | 9.8 |

**Analytical Recovery**

| Mouse serum | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| No. 1 | 0 | 5.933 | | |
| | 1.52 | 7.728 | 7.453 | 103.7 |
| | 4.55 | 10.202 | 10.483 | 97.3 |
| | 13.64 | 18.696 | 19.573 | 95.5 |
| No. 2 | 0 | 4.660 | | |
| | 1.52 | 5.854 | 6.180 | 94.7 |
| | 4.55 | 8.464 | 9.210 | 91.9 |
| | 13.64 | 15.779 | 18.300 | 86.2 |
| No.3 | 0 | 2.629 | | |
| | 1.52 | 3.943 | 4.149 | 95.0 |
| | 4.55 | 6.530 | 7.179 | 91.0 |
| | 13.64 | 14.148 | 16.269 | 87.0 |

| Mouse plasma | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| No.1 | 1.52 | 4.257 | 4.358 | 97.7 |
| | 4.55 | 6.577 | 7.388 | 89.0 |
| | 13.64 | 14.120 | 16.478 | 85.7 |
| No.2 | 0 | 2.843 | | |
| | 1.52 | 4.215 | 4.363 | 96.6 |
| | 4.55 | 6.569 | 7.393 | 88.9 |
| | 13.64 | 14.267 | 16.483 | 86.6 |
| No.3 | 0 | 2.855 | | |
| | 1.52 | 4.197 | 4.375 | 95.9 |
| | 4.55 | 6.915 | 7.405 | 93.4 |
| | 13.64 | 14.801 | 16.495 | 89.7 |

| Rat serum | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| No.1 | 0 | 4.908 | | |
| | 1.52 | 6.711 | 6.428 | 104.4 |
| | 4.55 | 9.888 | 9.458 | 104.5 |
| | 13.64 | 20.396 | 18.548 | 110.0 |
| No.2 | 0 | 5.462 | | |
| | 1.52 | 6.317 | 6.982 | 90.5 |
| | 4.55 | 10.117 | 10.012 | 101.0 |
| | 13.64 | 19.222 | 19.102 | 100.6 |

| | | | | |
|---|---|---|---|---|
| No.3 | 0 | 4.043 | | |
| | 1.52 | 5.194 | 5.563 | 93.4 |
| | 4.55 | 8.209 | 8.593 | 95.5 |
| | 13.64 | 16.777 | 17.683 | 94.9 |

| Rat plasma | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| No.1 | 0 | 6.755 | | |
| | 1.52 | 7.777 | 8.275 | 94.0 |
| | 4.55 | 11.546 | 11.305 | 102.1 |
| | 13.64 | 22.639 | 20.395 | 111.0 |
| No.2 | 0 | 6.567 | | |
| | 1.52 | 7.645 | 8.087 | 94.5 |
| | 4.55 | 10.816 | 11.117 | 97.3 |
| | 13.64 | 22.094 | 20.207 | 109.3 |
| No.3 | 0 | 4.463 | | |
| | 1.52 | 5.589 | 5.983 | 93.4 |
| | 4.55 | 8.824 | 9.013 | 97.9 |
| | 13.64 | 18.032 | 18.103 | 99.6 |

| Mouse urine | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| No.1 | 0 | 6.332 | | |
| | 1.52 | 8.340 | 7.852 | 106.2 |
| | 4.55 | 12.603 | 10.882 | 115.8 |
| | 13.64 | 22.998 | 19.972 | 115.2 |
| No.2 | 0 | 2.578 | | |
| | 1.52 | 4.476 | 4.098 | 109.2 |
| | 4.55 | 7.957 | 7.128 | 111.6 |
| | 13.64 | 18.517 | 16.218 | 114.2 |

| Tissue culture medium | Corticosterone added (ng/mL) | Observed (ng/mL) | Expected (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| RPMI-1640 | 0 | 2.379 | | |
| | 1.52 | 3.711 | 3.899 | 95.2 |
| | 4.55 | 6.821 | 6.929 | 98.4 |
| | 13.64 | 16.496 | 16.019 | 103.0 |
| RPMI-1640+ 10% FCS | 0 | 0.723 | | |
| | 1.52 | 2.031 | 2.243 | 90.5 |
| | 4.55 | 5.105 | 5.273 | 96.8 |
| | 13.64 | 14.881 | 14.363 | 103.6 |

**Dilution Test**

| Mouse serum | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| No.1 | 1 | 6.550 | | |
| | 2 | 3.368 | 3.275 | 102.8 |
| | 4 | 1.700 | 1.638 | 103.8 |
| | 8 | 0.936 | 0.819 | 114.3 |
| No.2 | 1 | 5.408 | | |
| | 2 | 2.707 | 2.704 | 100.1 |
| | 4 | 1.450 | 1.352 | 107.2 |
| | 8 | 0.808 | 0.676 | 119.5 |
| No.3 | 1 | 3.792 | | |
| | 2 | 1.968 | 1.896 | 103.8 |
| | 4 | 1.022 | 0.948 | 107.8 |
| | 8 | 0.526 | 0.474 | 111.0 |

| Mouse plasma | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| No.1 | 1 | 3.033 | | |
| | 2 | 1.532 | 1.517 | 101.0 |
| | 4 | 0.808 | 0.758 | 106.6 |
| | 8 | 0.430 | 0.379 | 113.4 |
| No.2 | 1 | 2.894 | | |
| | 2 | 1.559 | 1.447 | 107.7 |
| | 4 | 0.838 | 0.724 | 115.8 |
| | 8 | 0.436 | 0.362 | 120.5 |
| No.3 | 1 | 2.486 | | |
| | 2 | 1.121 | 1.243 | 90.2 |
| | 4 | 0.564 | 0.622 | 90.7 |
| | 8 | 0.308 | 0.311 | 99.1 |

| Rat serum | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| No.1 | 1 | 7.660 | | |
| | 2 | 3.414 | 3.830 | 89.1 |
| | 4 | 1.690 | 1.915 | 88.3 |
| | 8 | 0.895 | 0.958 | 93.5 |
| No.2 | 1 | 6.692 | | |
| | 2 | 3.201 | 3.346 | 95.7 |
| | 4 | 1.618 | 1.673 | 96.7 |
| | 8 | 0.765 | 0.837 | 91.5 |
| No.3 | 1 | 4.797 | | |
| | 2 | 2.302 | 2.399 | 96.0 |
| | 4 | 0.981 | 1.199 | 81.8 |
| | 8 | 0.583 | 0.600 | 97.2 |

| Rat plasma | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| No.1 | 1 | 6.674 | | |
| | 2 | 3.123 | 3.337 | 93.6 |
| | 4 | 1.666 | 1.669 | 99.9 |
| | 8 | 0.786 | 0.834 | 94.2 |
| No.2 | 1 | 8.323 | | |
| | 2 | 3.969 | 4.162 | 95.4 |
| | 4 | 1.831 | 2.081 | 88.0 |
| | 8 | 0.981 | 1.040 | 94.3 |
| No.3 | 1 | 4.635 | | |
| | 2 | 2.359 | 2.318 | 101.8 |
| | 4 | 1.186 | 1.159 | 102.4 |
| | 8 | 0.595 | 0.579 | 102.7 |

| Mouse urine | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| No.1 | 1 | 7.841 | | |
| | 2 | 3.642 | 3.921 | 92.9 |
| | 4 | 2.011 | 1.960 | 102.6 |
| | 8 | 1.095 | 0.980 | 111.7 |
| No.2 | 1 | 2.912 | | |
| | 2 | 1.479 | 1.456 | 101.6 |
| | 4 | 0.874 | 0.728 | 120.1 |
| | 8 | 0.536 | 0.364 | 147.3 |

| Tissue culture medium | Dilution ratio (1X ) | Observed (ng/mL) | Expected (ng/mL) | % of expected |
|---|---|---|---|---|
| RPMI-1640 | 1 | 2.710 | | |
| | 2 | 1.330 | 1.355 | 98.2 |
| | 4 | 0.652 | 0.678 | 96.2 |
| | 8 | 0.320 | 0.339 | 94.5 |
| RPMI1640+ | 1 | 0.866 | | |
| 10% FCS | 2 | 0.586 | 0.433 | 135.3 |
| | 4 | 0.240 | 0.217 | 110.9 |
| | 8 | | 0.108 | |

**Cross Reactivity**

Cross reactivities of the antibody used in the kit.

| Compound | Cross Reactivity (%) |
|---|---|
| Corticosterone | 100 |
| 11-Deoxycorticosterone | <15.5 |
| Progesterone | <5.9 |
| Androstenedione | <5.4 |
| Testosterone | <3.9 |
| Aldosterone | <1.7 |
| Cortisol | <0.5 |
| Cortisone | <0.4 |
| DHEA | <0.05 |
| Estradiol | 0 |
| Cholesterol | 0 |

**FOR RESEARCH USE ONLY**

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