Corticosterone ELISA

Cat. No.: RSCYK240R

1. INTRODUCTION

Corticosterone ($C_{21}H_{30}O_4$, also called 11β,21-Dihydroxyprogesterone, Reichstein's Substance H, or Kendall's Compound B) is, like cortisol and cortisone, a glucocorticoid hormone secreted from the cortex of adrenal gland. Corticosterone is derived from cholesterol through a series of enzymatically mediated steps and also serving as a precursor of aldosterone. It is a primary glucocorticoid in mice and rats and other animals (such as rabbits, birds, amphibians, and reptilians) in which the 17α-hydroxylase is supposed not to exist in 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 [(1)].

Corticosterone can be used as a non-invasive biomarker of stress study through the collection of urine and feces to avoid corticosterone increase of blood levels which is caused by normal invasive methods [(2)]. Corticosterone is also being studied in different fields such as impairment of long-term memory retrieval [(3)], chronic corticosterone elevation due to dietary restrictions [(4)] and response to burn injuries [(5)] etc.

Since most of corticosterone in blood is bound to a plasma protein called corticosteronebinding 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 EIA kit newly developed will be a quite useful tool for further development of corticosterone research.

	RSCYK240R Corticosterone EIA Kit		Contents
▼	The kit assay range: 0.21-50 ng/mL.	1)	Antibody Coated Plate
▼	The assay running time: 2 h. + 0.5 h.	2)	Corticosterone Standard
▼	Maximum measurable samples: 41 in duplicate	3)	HRP-Labeled Corticosterone
▼	Test sample: plasma, serum, urine, and cell or tissue culture supernatant etc.	4)	Specific Antibody
▼	The 96-well plate in the kit is consisted of 8-wells strips, so that the kit can be used dividedly in strips.	5)	TMB Substrate
▼	Intra-assay %CV: 2.5~4.7	6)	Reaction Stopping Solution
▼	Inter-assay %CV: 7.7~9.8.	7)	Buffer Solution
		8)	Sample Diluent
▼	Store all the components in the kit at 2-8°C.	9)	Concentrated Wash Solution
	The expiry date is stated on the package.	10)	Adhesive Foil

2. CHARACTERISTICS

This EIA kit is used for 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, practically no influences of other body fluids or physiological active substances coexisting in samples assayed.

Specificity
The specificity of this EIA kit is shown on page 11.

Assay Principle
This EIA kit for determination of corticosterone is based on a competitive enzyme immunoassay using combination of specific antibody to corticosterone and corticosterone- horseradish peroxidase (HRP) conjugate (HRP-labeled corticosterone) system. The 96 wells plate is coated with goat anti rabbit IgG, to which corticosterone standard 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.

3. COMPOSITION

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiter plate	1 Plate (96 wells)	Goat anti rabbit IgG
2.	Corticosterone Standard	Lyophilized powder	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)	3,3',5,5'-Tetramethylbenzidine (TMB)
6.	Reaction Stopping solution	Liquid	1 Bottle (12 mL)	1M Sulfuric acid
7.	Buffer solution	Liquid	1 Bottle (10 mL)	BSA-containing PBS buffer
8.	Sample Diluent	Liquid	1 Bottle (50 mL)	A specially formulated displacer of CBG
9.	Concentrated Wash Solution liquid	Liquid	1 Bottle (25 mL)	Concentrated saline
10	Adhesive foil		2 Sheets	

4. METHOD

Equipment required

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system (optional)
3. Micropipettes for volumes between 10 µL –1000 µL
4. Multi-channel pipettes for 8 or 12 wells and the tips
5. Glass test tubes for preparation of standard and sample solutions
6. A microplate shaker (210-240 rpm)
7. Graduated cylinder (500 mL or 1,000 mL)
8. Distilled or deionized water

Preparatory work

1. Preparation of corticosterone standard solution: Reconstitute lyophilized Corticosterone Standard (50 ng/vial) with 1 mL of Sample Diluent, which affords 50 ng/mL standard solution. The reconstituted corticosterone standard solution (0.2 mL) is diluted with 0.4 mL of Sample Diluent that yields 16.67 ng/mL standard solution. Repeat the same dilution to make standard solution of 5.56, 1.85, 0.62, and 0.21 ng/mL, respectively. Sample Diluent is used as 0 ng/mL.
2. Preparation of HRP-labeled corticosterone solution: Take 0.25 mL of HRP-Labeled Corticosterone from the labeled vial to dilute with 7 mL of Buffer Solution completely.
3. Dilution of Wash Solution Concentrated: Dilute one bottle of Wash Solution Concentrated (25 mL) to 500 mL with distilled or deionized water.
4. 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 still for 10 minutes at room temperature.
2. For mouse/rat urine: Dilute 10 µL of urine sample (40-100 folds) with Sample Diluent in a test tube. Mix the diluted solution and allow it to stand still 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 still 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.

Assay procedure

1. Before starting assay, bring all the reagents, except test samples, to room temperature (20-30°C).
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 it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.

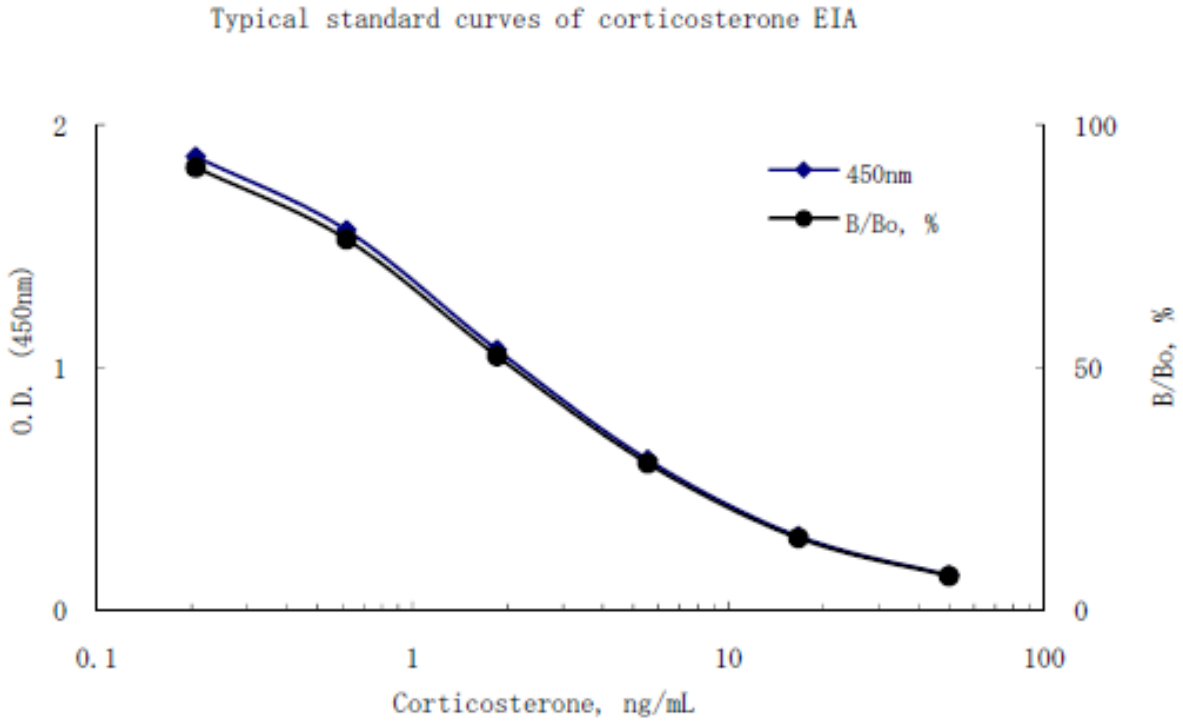
3. Pipet 100 µL of corticosterone standard solutions (0, 0.21, 0.62, 1.85, 5.56, 16.67, and 50 ng/mL) or diluted samples, after vortexed, into appropriate wells. Add 50 µL of HRP-labeled corticosterone solution into each well, and finally add 50 µL of Specific Antibody into each well.
4. Cover the plate with adhesive foil and incubate it on a shaker at 210-220 rpm at room temperature for 2 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 it onto an absorbent surface, such as paper toweling, to ensure blotting free of most 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 Reaction Stopping Solution into each well to stop color reaction.
9. Read the optical absorbance of the wells at 450 nm.
10. 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 standard solutions or their percent bound to maximum binding wells (0 ng/mL) and plot a standard curve on a semi-logarithmic graph paper (abscissa: concentrations of standard; ordinate: optical density or bound%). Use the average optical density or bound% of each sample to determine the corresponding value by simple interpolation from the standard curve. The results should be multiplied by the diluting factor to obtain the actually concentrations for undiluted unknown samples.

5. NOTES

1. It is recommended that serum or plasma samples should be used as soon as possible after collection. If the sample is tested later, they should be aliquoted and frozen below –30°C (for long term storage, in a –80°C deep freezer). Avoid repeated freezing and thawing of samples.
2. Corticosterone standard solutions and HRP-labeled corticosterone solution should be prepared immediately before use. If the kit used dividedly, the rests of the reconstituted corticosterone standard solution (50 ng/mL), HRP-labeled corticosterone solution and other reagents except wash solution and stopping solution should be stored at 4°C and used within 2 weeks. Diluted standard solutions, except 50 ng/mL of standard solution, should not be reused for another assay.
3 The recommended diluting ratio for mouse or rat sample is 20-100 folds, but corticosterone levels significantly differ among animals and also show marked circadian variation even in the same individual. Therefore, optimal dilution test should be run when handling test samples of species other than mice and rats. In addition, since progesterone level increases significantly in pregnant animal, the cross reactivity of the steroid should be considered (refer to page 10 <Cross reactivity>) when such samples are tested.
4. Incomplete washing of the microplate will interfere with assay precision. If a microplate washer is not available, completely aspirate the solutions in the wells of 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 final wash.
5. As pipetting operations may affect precision of the assay, pipet corticosterone standard solutions or samples precisely into the wells of assay plate. In addition, use clean test tubes or vessels in assay and a new tip for each standard diluting process and for each sample or standard solution pipetting to avoid cross contamination.
6. Perform all the determination in duplicate.
7. To quantitate accurately, always run a standard curve for each assay.
8. Color reaction should be carried out under the light proof condition.
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
10. Protect the reagents from strong light (e.g. direct sunlight) during storage and assay.

11. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.

6. PERFORMANCE CHARACTERISTICS



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 [6].

$$\text{Sensitivity (ng/mL)} = \frac{2 \times \text{SD of the Zero Standard} \times 0.21 \text{ ng/mL}}{(\text{Optical Density of 0ng/mL} - \text{Optical Density of 0.21ng/mL})}$$

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

(Samples were diluted as indicated dilution method before recovery test)

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	
	4	1.690	1.915	
	8	0.895	0.958	
No. 2	1	6.692		
	2	3.201	3.346	
	4	1.618	1.673	
	8	0.765	0.837	
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	Corticosterone added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)

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	Corticosterone added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
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+ 10% FCS	1	0.866		
	2	0.586	0.433	135.3
	4	0.240	0.217	110.9
	8		0.108	

(Samples were diluted as indicated dilution method before dilution test)

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

7. STABILITY AND STORAGE

Storage Store all the components in the kit at 2°C - 8°C.

Shelf life The Kit is stable under the storage condition for 12 months from the date of manufacture.
The expiry date is stated on the label of package.

Package For 96 tests per one kit including standards.

8. REFERENCES

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4. Kitaysky AS, Kitaiskaiya EV et al: Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J Comp Physiol B: Regulatory, Integrative and Comparative Physiology* 171:701-709, 2001
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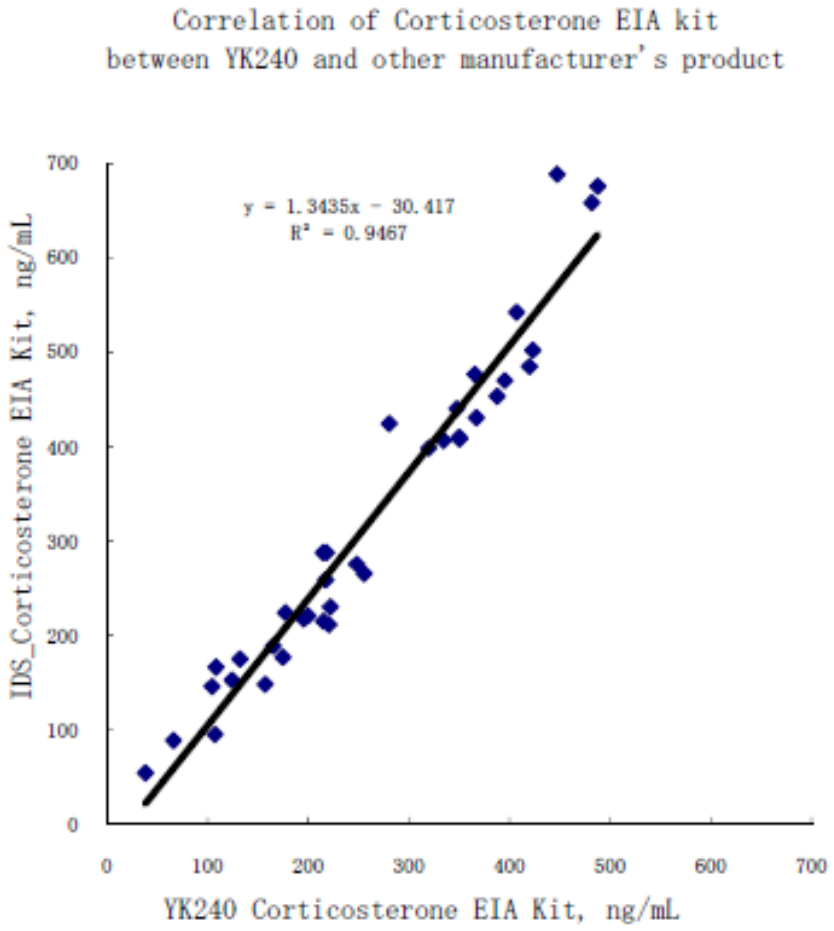
9. APPENDIX

The RSCYK240R Corticosterone EIA kit has been compared to other commercially available Corticosterone EIA kit. Thirty-seven samples of mouse and

rat plasma or serum were assayed and linear regression analysis of the results yielded as shown in the graph.



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