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Instructions for use

Corticosterone rat / mouse ELISA

REF AR E-8100

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Corticosterone rat/mouse ELISA

1. INTRODUCTION

1.1 Intended Use

The **Corticosterone rat/mouse ELISA** is a competitive immunoassay for the measurement of corticosterone in rat and mouse serum or plasma. For research use only. Not for use in diagnostic procedures.

1.2 Summary and Explanation

Corticosterone is secreted by the adrenal cortex under control of the pituitary hormone ACTH via a negative feedback mechanism. It is the most abundant circulating steroid in rats, since rodents are not able to synthesize Cortisol, the major glucocorticoid in human, as a result of lacking the enzyme C17-Hydroxylase.
Corticosterone has a wide range of activities in rodents. It regulates carbohydrate, protein and fat metabolism. It has also an influence on the hemopoietic system and reduces the total number of lymphocytes and eosinophils, but to a lesser extent than cortisol. In contrast to cortisol, corticosterone has only minimal anti-inflammatory activity.
Corticosterone level in nocturnal animals like rats exhibit a distinct circadian variation with peak values in the latter portion of the day, followed by a nadir in the morning (1) and is believed to play an important role in sleep-wake cyclus (2). This is in contrast to diurnal mammals, where peak concentrations of glucocorticoids are found in the morning. Enhanced corticosterone release by female compared to male rats under basal and stress conditions has been observed (6).
Determination of corticosterone in rats is of interest to facilities conducting neurophysiological research, to academic institutions and to pharmaceutical companies with drug research departments. Drugs that influence the endocrine system can increase or reduce corticosteroid production in the adrenal cortex. Rat serum corticosterone is therefore an ideal indicator of the side effects of a potential therapeutic agent. The same constellations of effects seen in rats are generally seen in human. Plasma corticosterone in rats is often used in connection with ACTH measurement as a stress indicator (3, 4). The effects of chronic stress on the function of the hypothalamic-pituitary-adrenocortical system are age-dependent. Recent studies suggest that aging increases basal but not stress induced levels of corticosterone in the brain (5).

2. PRINCIPLE

The Corticosterone rat/mouse ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. An unknown amount of corticosterone present in the sample and a defined amount of corticosterone conjugated to horseradish peroxidase compete for the binding sites of corticosterone antiserum coated to the wells of a microplate. After incubation on a shaker the microplate is washed four times. After addition of the substrate solution the concentration of corticosterone is inversely proportional to the optical density measured.

3. WARNINGS AND PRECAUTIONS

1. This kit is for research use only. Not for use in diagnostic procedures.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21 – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.

14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents Provided

AR E-8131 96 **Microtiterplate** - Ready to use

Content: 12 x 8 (break apart) strips with 96 wells; wells coated with anti-corticosterone antibody

Standards - ready to use.

Cat. no.	Symbol	Standards	Concentration	Volume / Vial
AR E-8101	STANDARD A	**Standard A**	0 ng/ml	0.3 ml
AR E-8102	STANDARD B	**Standard B**	15 ng/ml	0.3 ml
AR E-8103	STANDARD C	**Standard C**	50 ng/ml	0.3 ml
AR E-8104	STANDARD D	**Standard D**	185 ng/ml	0.3 ml
AR E-8105	STANDARD E	**Standard E**	640 ng/ml	0.3 ml
AR E-8106	STANDARD F	**Standard F**	2250 ng/ml	0.3 ml

AR E-8113 INC-BUFF **Incubation Buffer** - Ready to use

Volume: 1 x 11 ml

AR E-8140 CONJUGATE **Enzyme Conjugate** - Ready to use

Content: Corticosterone conjugated to horseradish peroxidise.

Volume: 1 x 7 ml

AR E-0055 SUBSTRATE **Substrate Solution** - Ready to use

Content: contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

Volume: 1 x 22 ml

AR E-0080 STOP-SOLN **Stop Solution** - Ready to use

Content: contains 2 N Hydrochloric Acid solution.

Volume: 1 x 7 ml

Hazards identification:

H290 May be corrosive to metals.
H314 Cause severe skin burns and eye damage.
H335 May cause respiratory irritation.

AR E-0030 WASH-CONC 10x **Wash Solution** - 10 x concentrated

Volume: 1 x 50 ml

See "Preparation of Reagents"

Note: Additional Standard A for sample dilution is available upon request.

4.2 Materials Required but not Provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Microplate mixer operating more than 600 rpm
- Calibrated variable precision micropipettes (10 µl, 50 µl, 100 µl, 200 µl).
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Reagent Preparation

All reagents should be at room temperature before use.

Wash Solution:
Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for at least 3 months at room temperature.

4.4 Storage Conditions

When stored at 2°C to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°-8°C. After first opening the reagents are stable for 30 days if used and stored properly.
Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

4.5 Disposal of the Kits

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, the manufactuer has to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SAMPLE

For determination of Corticosterone rat/mouse serum and plasma can be used. The procedure calls for 10 µl matrix per well. The samples should assay immediately or aliquot and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain rat/mouse Corticosterone concentrations higher than the highest standard (2250 ng/ml) should be diluted with the Standard A before assay. The additional dilution step has to be taken into account for the calculation of the results.

Please note: The use of plasma as specimen can result in a diminished precision of this assay.

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard and sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.
- For internal quality control we suggest to use **Rat Control Set (AR K-8000) For more information please contact the manufacturer.**

6.2 Assay Procedure

Each run must include a standard curve.

1.	Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2.	Dispense **10 µl** of each **Standard, Sample and Control** with new disposable tips into appropriate wells
3.	Dispense **100 µl** of **Incubation Buffer** into each well.
4.	Add **50 µl Enzyme Conjugate** into each well
5.	Incubate for **2 hours** at room temperature on a microplate mixer (> 600 rpm) **Important Note:** Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6.	Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7.	Add **200 µl** of **Substrate Solution** to each well.
8.	Incubate without shaking for **30 minutes** in the dark at room temperature.
9.	Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
10.	Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Conversion to SI units:

Corticosterone (ng/ml) x 2.886 = nmol/l

6.3.1 Example of Typical Standard Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard		Absorbance Units
Standard A	(0 ng/ml)	3.004
Standard B	(15 ng/ml)	2.817
Standard C	(50 ng/ml)	2.505
Standard D	(185 ng/ml)	1.620
Standard E	(640 ng/ml)	0.723
Standard F	(2250 ng/ml)	0.297

7. EXPECTED NORMAL VALUES

In order to determine the normal range of serum corticosterone in rat, samples of male and female rats were collected in the morning (7.00 – 9.00 a.m.) as well as in the late afternoon (5.00 – 6.00 p.m.) and analyzed using the Corticosterone rat/mouse ELISA kit. The following ranges are calculated with the results of this study.

	Range (ng/ml) Morning	Range (ng/ml) Late afternoon
Male rats ♂	n.d. – 11.4	172.6 – 245.4
Female rats ♀	53.9 – 332.1	292.5 – 819.0

n.d. non detectable

In further studies serum samples of 23 mice were collected between 11.00 a.m. and 2.00 p.m. und analyzed in a similar manner.

	Range (ng/ml)
Male mice ♂	47 – 159

It is recommended that each laboratory establish its own normal range since corticosterone levels can vary due to handling and sampling techniques.

8. PERFORMANCE CHARACTERISTICS

8.1 Analytical Sensitivity

The lowest analytical detectable level of corticosterone that can be distinguished from the Standard A is 6.1 ng/ml at the 2SD confidence limit.

8.2 Specifictiy

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to corticosterone.

Steroid	% Cross reaction
Aldosterone	0.3
Cortisol	2.3
11-Deoxycorticosterone	12.5
Dehydroepiandrosterone	<0.1
Estrone	<0.1
Estradiol	<0.1
17-Hydroxyprogesterone	<0.1
Progesterone	6.2
Testosterone	<0.1
5α-Dihydrotestosterone	<0.1
5α-Androstane	<0.1
Androstenedione	<0.1
Androsterone	<0.1
Pregnenolone	1.1

8.3 REPRODUCIBILITY

8.3.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

Mean (ng/ml)	62.8	126.0	271.4
SD	5.6	9.2	16.0
CV (%)	8.9	7.3	5.9
n =	20	20	20

8.3.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples.

Mean (ng/ml)	59.3	113.2	257.4
SD	4.3	9.3	19.4
CV (%)	7.2	8.2	7.5
n =	10	10	10

8.4 Recovery

Using a steroid-free serum a spiking solution was prepared (5000 ng/mL). Aliquots of 20, 40, 60 and 80 µL, respectively, were spiked into 480, 460, 440 µL and 420 µL of three rat serum pools leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by the Corticosterone rat/mouse ELISA Procedure.

Serum	Spiking (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	-	29.1	./.	./.
	200	204.1	229.1	89%
	400	444.0	429.1	103%
	600	629.5	629.1	100%
2	-	122.5	./.	./.
	200	265.4	322.5	82%
	400	497.6	522.5	95%
	600	672.0	722.5	93%
3	-	137.3	./.	./.
	400	572.8	537.3	107%
	600	883.1	737.3	120%
	800	1068.5	937.3	114%

8.5 Linearity

Four native serum samples were assayed undiluted and diluted with the standard matrix.

Serum	Dilution	Observed (ng/mL)	Expected (ng/mL)	Linearity (%)
1	native	650,0	./.	./.
	1 in 2	304,5	325,0	94%
	1 in 4	157,6	162,5	97%
	1 in 8	67,2	81,2	83%
2	native	405,7	./.	./.
	1 in 2	210,2	202,9	104%
	1 in 4	108,5	101,4	107%
	1 in 8	55,7	50,7	110%
3	native	477,9	./.	./.
	1 in 2	235,4	239,0	98%
	1 in 4	107,1	119,5	90%
	1 in 8	48,1	59,7	81%
4	native	415,5	./.	./.
	1 in 2	186,3	207,8	90%
	1 in 4	79,7	103,9	77%
	1 in 8	37,7	51,9	73%

9. LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Drug Interferences

Until now no substances (drugs) are known influencing the measurement of rat or mouse corticosterone in serum. Lipemic and haemolysed samples can cause false results.

10. LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11. REFERENCES

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!