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Instructions for use

Corticosterone rat/mouse ELISA

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INTRODUCTION

INTENDED USE

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

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).

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.

WARNINGS AND PRECAUTIONS

1. For professional use only.
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. Do not mix reagents of different lots. Do not use expired reagents.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch and used in the frame provided.
5. Avoid contact with Stop Solution. It may cause skin irritation and burns.

REAGENTS

Reagents provided:

AR E-8131 MTP 96 **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;
Wells coated with polyclonal rabbit anti-corticosterone antibody.

Calibrators - ready to use.

Cat. no.	Symbol	Calibrator	Concentration	Volume/Vial
AR E-8101	STANDARD A	**Calibrator 0**	0 ng/ml	0.3 ml
AR E-8102	STANDARD B	**Calibrator 1**	15 ng/ml	0.3 ml
AR E-8103	STANDARD C	**Calibrator 2**	50 ng/ml	0.3 ml
AR E-8104	STANDARD D	**Calibrator 3**	185 ng/ml	0.3 ml
AR E-8105	STANDARD E	**Calibrator 4**	640 ng/ml	0.3 ml
AR E-8106	STANDARD F	**Calibrator 5**	2250 ng/ml	0.3 ml

AR E-8113 INC-BUFF **Incubation Buffer,** 1 vial 11 ml, ready to use;

AR E-8140 CONJUGATE **Enzyme Conjugate**, 1 vial, 7 ml, ready to use;
Corticosterone conjugated to horseradish peroxidise.

AR E-0055 SUBSTRATE **Substrate Solution**, 1 vial, 22 ml each, ready to use;
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

AR E-0080 STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;
contains 2 N Hydrochloric Acid solution.

AR E-0030 WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10X concentrated);
see „Preparation of Reagents".

Note: Additional Calibrator 0 for sample dilution is available upon request.

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

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.

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°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Take

SPECIMEN

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 calibrator (2250 ng/ml) should be diluted with the zero calibrator 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.

ASSAY PROCEDURE

General Remarks

- All reagents and specimens 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.
- For internal quality control a **Rat Control Set** is available upon request.

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 **Calibrator and Sample** 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.
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.

Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and patient 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 calibration curve.

Example of Typical Calibrator Curve

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

Standard	Absorbance Units
Calibrator 0 (0 ng/ml)	3.004
Calibrator 1 (15 ng/ml)	2.817
Calibrator 2 (50 ng/ml)	2.505
Calibrator 3 (185 ng/ml)	1.620
Calibrator 4 (640 ng/ml)	0.723
Calibrator 5 (2250 ng/ml)	0.297

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 am) as well as in the late afternoon (5.00 – 6.00 pm) 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 am and 2.00 pm 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.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

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

Specificity

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

Reproducibility

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

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

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%

Linearity

Four native serum samples were assayed undiluted and diluted with the calibrator 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%

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.

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.

LEGAL ASPECTS

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 DEMEDITEC.

Liability

3. De Souza EB & van Loon GR (1982): Stress induced inhibition of the plasma corticosterone response to a subsequent stress in the rat: A nonadrenocorticotropin-mediated mechanism. *Endocrinology* **110**, 1: 23-33
4. Kant GJ, Leu JR, Anderson SM & Mougey EH (1987): Effects of chronic stress on plasma corticosterone, ACTH and prolactin. Physiology & Behaviour **40**, 6: 775-779
5. Garrido P, de Blas M, Del Arco A, Segovia G & Mora F (2010): Aging increases basal but not stress-induced levels of corticosterone in the brain of the awake rat. *Neurobiol Aging*. 2010 Apr 21
6. Handa RJ, Burgess LH, Kerr JE, O'Keefe JA (1994): Gonadal Steroid Hormone Receptors and Sex Differences in the Hypothalamo-Pituitary-Adrenal Axis. *Hormon. Behav*. **28** (4): 464-76