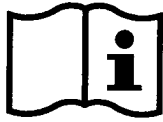


# Product information

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User's Manual

## Corticosterone rat/mouse ELISA

**RUO**

**REF**

**DEV9922**



**96 Wells**

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## 1 INTRODUCTION

### 1.1 INTENDED USE

The **DEMEDIATEC 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.

### 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 **DEMEDIATEC 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. 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.
6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
7. Change pipette tips between samples and reagents to avoid carry over contamination.
8. 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.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

11. Assay reagents contain Proclin against microbial growth. In case of contact with eyes or skin, flush immediately with water.
12. All reagents should be at room temperature (21-26°C) before use. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
13. TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

## 4 REAGENTS

### 4.1 REAGENTS PROVIDED

1. **SORB MT Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;  
Wells coated with polyclonal rabbit anti-corticosterone antibody.
2. **CAL 0 Calibrator 0**, 1 vial, 0.3 ml, ready to use
3. **CAL 1-5 Calibrator 1-5**, 5 vials, 0.3 ml each, ready to use;  
Concentrations: 15 – 50 – 185 – 640 – 2250 ng/ml
4. **INC BUF Incubation Buffer**, 1 vial 11 ml, ready to use;
5. **ENZ CONJ Enzyme Conjugate**, 1 vial, 7 ml, ready to use;  
Corticosterone conjugated to horseradish peroxidase.
6. **SUB TMB Substrate Solution**, 1 vial, 22 ml, ready to use;  
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
7. **STOP SOLN Stop Solution**, 1 vial, 7 ml, ready to use;  
contains 2 N Hydrochloric Acid solution.
8. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (10X concentrated);  
see „Preparation of Reagents“.

**Note:** Additional Calibrator 0 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°C to 8°C. 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, DEMEDITEC have 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 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.

## 6 ASSAY PROCEDURE

### 6.1 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.
- Respect the incubation times as stated in this instructions for use.
- For internal quality control we suggest to use **Rat Control Set coded DEV99RC**. For more information please contact DEMEDITEC.

### 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 **Calibrator, 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 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.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator 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 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

### 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 DEMEDITEC 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 Zero Calibrator is 6.1 ng/ml at the 2SD confidence limit.

## 8.2 SPECIFICITY

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

<b>Steroid</b>	<b>% Cross reaction</b>
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
5 $\alpha$ -Dihydrotestosterone	<0.1
5 $\alpha$ -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:

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

### 8.3.2 Inter-Assay

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

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

## 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 DEMEDITEC.

### 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

1. D'Agostino J, Vaeth GF & Henning SJ (1982): Diurnal rhythm of total and free concentration of serum corticosterone in the rat. *Acta Endocrinologica* **100**, Vol. 1, 85-90.
2. Vázquez-Palacios G et al. (2001): Further definition of the effect of corticosterone on the sleep-wake pattern in the male rat. *Pharmacol. Biochem. Behav.* **70**: 305-310
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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

## SYMBOLS USED WITH DEMEDITEC ELISA

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consultez le Mode d'emploi	Consulte las Instrucciones	Consulti le istruzioni
	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Référence	No de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
<i>Content</i>	Content	Inhalt	Contenu	Contenido	Contenuto
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
<i>Microtiterwells</i>	Microtiterwells	Mikrotiterwells	Plaques de microtitration	Pocillos de la Microplaca	Micropozzetti
<i>Antiserum</i>	Antiserum	Antiserum	Antisérum	Antisero	Antisiero
<i>Enzyme Conjugate</i>	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
<i>Enzyme Complex</i>	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
<i>Substrate Solution</i>	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
<i>Stop Solution</i>	Stop Solution	Stopplösung	Solution d'arrêt	Solución de paro	Soluzione d'arresto
<i>Zero Standard</i>	Zero Standard	Nullstandard	Standard 0	Standard 0	Standard zero
<i>Standard</i>	Standard	Standard	Standard	Calibrador	Standard
<i>Control</i>	Control	Kontrolle	Contrôle	Control	Controllo
<i>Assay Buffer</i>	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
<i>Wash Solution</i>	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
<i>1N NaOH</i>	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
<i>1 N HCl</i>	1 N HCl	1 N HCl	1N HCl	1 N HCl	
<i>Sample Diluent</i>	Sample Diluent	Probenverdünnungsmedium	Solution pour dilution de l'échantillon		Diluyente dei campioni
<i>Conjugate Diluent</i>	Conjugate Diluent	Konjugatverdünnungsmedium	Solution pour dilution du conjugué		Diluyente del tracciante

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Conformidade com as normas europeias	Europæisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Instruções de uso	Brugermanual	Användar manual	Εγχειρίδιο χρήστη
<b>IVD</b>	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
<b>REF</b>	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
<b>LOT</b>	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
<b>RUO</b>				
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
<i>Distributed by</i>				
<i>Content</i>	Conteúdo	Indhold	Innehåll	Περιεχόμενο
<i>Volume/No.</i>	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..
<i>Microtiterwells</i>	Alvéolos de microtitulação	Mikrotiterbrønde	Brunnar i Mikrotiterplatta	Πηγαδάκια Μικροπιλοδοτήσεως
<i>Antiserum</i>	Anti-soro	Antiserum	Antiserum	Αντιορός
<i>Enzyme Conjugate</i>	Conjugado enzimático	Enzymkonjugat	Enzymkonjugat	Συζευγμένο ενζυμο
<i>Enzyme Complex</i>	Complexo enzimático	Enzymkompleks	Enzymkomplex	Σύμπλοκο ενζύμου
<i>Substrate Solution</i>	Solução de substrato	Substratopløsning	Substratlösning	Διάλυμα υποστρώματος
<i>Stop Solution</i>	Solução de paragem	Stopopløsning	Stopp lösning	Διάλυμα τερματισμού
<i>Zero Standard</i>	Padrão zero	Standard 0	Standard 0	Πρότυπο Μηδέν
<i>Standard</i>	Calibrador	Standard	Standard	Πρότυπα
<i>Control</i>	Controlo	Kontrol	Kontroll	Έλεγχος
<i>Assay Buffer</i>	Tampão de teste	Assay buffer	Assay Buffer	Ρυθμιστικό Διάλυμα Εξέτασης
<i>Wash Solution</i>	Solução de lavagem	Vaskebuffer	Tvätt lösning	Διάλυμα πλύσεως
<i>1N NaOH</i>	1N NaOH	1N NaOH	1N NaOH	1N NaOH
<i>1 N HCl</i>	1 N HCl	1 N HCl	1 N HCl	1 N HCl
<i>Sample Diluent</i>				
<i>Conjugate Diluent</i>				