Mouse/Rat Corticosterone ELISA Kit

Enzyme Immunoassay for the quantification of Mouse/Rat Corticosterone in Mouse or Rat serum and plasma.

Catalog number: ARG80652

Package: 96 wells

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INTRODUCTION

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 and is believed to play an important role in sleep-wake cyclus. 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.

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.

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.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Corticosterone has been pre-coated onto a microtiter plate. Endogenous Corticosterone of a sample competes with a Corticosterone -horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Corticosterone in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of Corticosterone in the patient sample. Corticosterone concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard 0	0.3 ml (ready to use)	4°C
Standard 1-5 (15, 50, 185, 640, 2250 ng/ml)	5 x 0.3 ml (ready to use)	4°C
Incubation Buffer	11 ml (ready to use)	4°C
HRP conjugated Corticosterone	7 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml (ready to use)	4°C (Protect from light)
STOP solution	7 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Orbital microplate shaker: 3 mm (0.1118 in) 600 ± 10 rpm or 19 mm (0.75 in) 170 ± 10 rpm.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. 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.
- Opened reagents must be stored at 2°-8°C. After first opening the reagents are stable for 30 days if used and stored properly.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- All kit reagents and specimens should be brought to room temperature (21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water) The diluted Wash buffer is stable for at least 3 months at room temperature.
- Samples: If the results of samples are out of the measuring range, or the samples are expected to contain Corticosterone higher than the highest standard (2250 ng/ml), the samples can be diluted with Standard 0 and re-assay the samples. The dilution factor should be taken into account for the result calculation.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **10 µl** of **standards and samples** in duplicate into sample wells.
3. Add **100 µl** of **Incubation Buffer** into each well.
4. Add **50 µl** of **HRP conjugated Corticosterone** into each well. Incubate for **2 hours at RT** on a **microplate shaker (600 rpm)**.

   Important Note:

   Optimal reaction in this assay is markedly dependent on shaking of the microplate.
5. Aspirate each well and wash, repeating the process 3 times for a total **4 washes.** Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **200 µl** of **TMB substrate** to each well. Incubate for **30 minutes at room temperature** in dark (without shaking).
7. Add **50 µl** of **Stop Solution** to each well.
8. Read the OD with a microplate reader **at 450 nm** immediately. It is recommended to read the wells within 15 minutes after adding Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic or linear 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 method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)

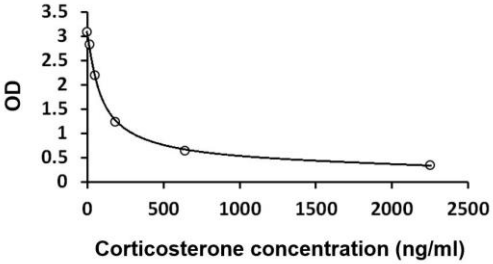
6. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted with zero standard. For the calculation of the concentrations, this dilution factor has to be taken into account.

7. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	0	1	2	3	4	5
Corticosterone (ng/ml)	0	15	50	185	640	2250
Corticosterone (nmol/L)	0	43.29	144.3	533.91	1847.0	6493.5
Conversion	Corticosterone (ng/ml) x 2.886 = Corticosterone (nmol/L)					

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



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 Mouse/Rat Corticosterone 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

In further studies serum samples of 23 mice were collected between 11:00 am and 2:00 pm and 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.

QUALITY ASSURANCE

Specificity

Steroid	Crossreactivity (%)
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
Pregnolone	1.1
Androstenedione	< 0.1
Androsterone	< 0.1

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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.3% and inter-assay precision was 7.6%.

Recovery

82-120%

Linearity

77-110%