Rat, mouse Corticosterone ELISA KIT

(REF: EK-548)

For Research Use Only. Not for use in diagnostic procedures.

The rat, mouse Corticosterone ELISA system provides direct quantitative *in vitro* determination of rat and mouse Corticosterone in plasma and serum. Rat and mouse Corticosterone can be measured in the range 1.5-800 ng/mL. Each kit contains materials sufficient for 96 determinations permitting the construction of one standard curve and the assay of 40 unknowns in duplicate.

Introduction

The adrenal cortex produces 3 types of steroid hormones: glucocorticoids, mineralocorticoids and androgens or oestrogens. The glucocorticoids, which include corticosterone and cortisol, have important effects on carbohydrate and proteinmetabolism in mammals. Glucocorticoids are mainly produced in the inner layers of the adrenal cortex, the zona fasciculata and zona reticularis, as they contain the appropriate steroidogenic enzymes.

Synthesis of all adrenal steroids starts with the conversion of the common precursor cholesterol into pregnenolone and this is the main rate-limiting step. Corticosterone (MW346.5) is the main secreted glucocorticoid in the rat and rabbit while in man, monkey and sheep cortisol is the main secreted product. Cow,ferret, dog and cat secrete equal mixtures of both. Interestingly, cortisol and corticosterone are found in different biosynthetic pathways in the adrenal cortex. A characteristic 17.-hydroxylation of pregnenolone eventually leads to cortisol while corticosterone is one product in the pathway leading to the mineralocorticoid aldosterone. However, corticosterone possesses clear glucocorticoid activity and this is the main function in the rat.

Corticosterone is transported in the blood bound to a specific corticosteroid-binding globulin (CBG) (transcortin) which serves as a carrier and is in equilibrium with free steroid which is the active molecule. The mode of action of corticosterone is, as usual for steroids, that after interacting with cytosolic receptors the steroidreceptor complex binds to nuclear DNA to initiate transcription of specific genes.

Corticosterone stimulates hepatic gluconeogenesis, increases hepatic glycogen content and blood glucose levels, promotes lipolysis and has a catabolic effect leading to a negative nitrogen balance. It is one of the mediators of the stress response to which these actions, which tend to oppose those of insulin, are an adaptation.

Glucocorticoid secretion by the adrenal is stimulated by adrenocorticotrophin (ACTH) which acts to increase the rate of conversion of cholesterol to pregnenolone. Corticosterone acts in a negative feedback loop to inhibit the secretion of ACTH and corticotrophin releasing hormone (CRH) by the pituitary and hypothalamus respectively. This regulatory system is also influenced by other factors such as diurnal rhythm and stress. In the rat corticosterone levels are at a peak in late afternoon and evening. Corticosterone concentrations can also be influenced by handling stress, anaesthesia, sex, and age in the rat.

Principle of method

The assay is based on the competition between unlabelled corticosterone and a fixed quantity of HRP-labelled corticosterone for a limited number of binding sites on a corticosterone specific antibody. With fixed amounts of antibody and labelled ligand, the amount of labelled ligand bound by the antibody will be inversely proportional to the concentration of added non- labelled ligand.

This assay is based on a one-step procedure. The standards and samples are incubated in streptavidin coated wells with biotin labelled antibody and HRP-labelled corticosterone. During a 1-hour incubation period with continuous agitation the antibody - antigen complex is developed and immobilized on the reactive surface of wells. After the incubation period the reaction mixture is washed repeatedly.

After the addition of a ready-to-use tetramethyl-benzidine (TMB) peroxide substrate the signal is measured in an ELISA photometer at 450 nm wavelength.

The concentration of antigen is inversely proportional to the optical density measured in the wells. The unknown concentration of corticosterone in samples is read off a calibration curve constructed by plotting binding values against a series of calibrators containing known amount of corticosterone.

Contents of the kit

1. 1 bottle CONJUGATE (11 mL), ready to use, containing corticosterone conjugated to horseradish peroxidase in buffer with blue dye.
Store at 2-8 °C.

2. 8 vials STANDARDs (S0-S7), (S0 3 mL, S1-S7 0.5 mL per vial), ready to use, containing 1.5 (S1), 5 (S2), 20 (S3), 80 (S4), 200 (S5), 400 (S6) and 800 (S7) ng/mL corticosterone in serum with 0.1 % sodium azide.

Store at -20 °C upon arrival for long term. If used within 2 weeks store at 2-8 °C.

3. 1 bottle ANTISERUM (11 mL), ready to use, containing anti-corticosterone antibodies in buffer with 0.1 % sodium azide**.**

Store at 2-8 °C.

4. 1 bottle SUBSTRATE (25 mL), ready to use, in brown plastic bottle. Do not expose to direct light!
Store at 2-8 °C.

5. 1 piece MICROTITER PLATE, ready to use. 12 strips, packed in an air-tight foil.
Store at 2-8 °C.

6. 1 bottle WASH BUFFER CONCENTRATE (20 mL), with 0.1 % merthiolate.

Store at 2-8 °C.
See *Preparation of reagents.*

7. 1 bottle STOP REAGENT (6 mL), ready to use, 1M sulfuric acid.
Store at 2-8 °C.

Plate map
Cover sticks
Pack leaflet

Materials, tools and equipment required

Precision pipettes with disposable tips (25, 100, 200 and 300 µL), distilled water, vortex mixer, shaker, plastic trays (separate for each reagent), absorbent tissue, ELISA photometer.

Recommended tools and equipment
Repeating pipettes, multi-channel ELISA pipettes.

Specimen collection

This section is provided for guidance only. It remains the investigator's responsibility to validate the chosen sample collection technique.

Blood samples
It is advised that if measurements are to be made on plasma samples, blood should be collected into tubes containing EDTA. Blood should be centrifuged immediately to remove cells and the plasma stored below -15°C prior to analysis. Serum samples can also be assayed with this kit.

Samples may need to be diluted due to the high rat, mouse corticosterone plasma/serum concentration. The zero standard, S0 may be used for this purpose.

The present kit is suitable for direct determination of corticosterone in serum/plasma samples. There is no need to displace corticosterone from binding globulins prior to assay.

Preparation of reagents, storage

Storage: *see Contents of the kit*. At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label.

Preparation: Equilibrate all reagents and samples to room temperature prior to use.

Wash buffer: Add the wash buffer concentrate (20 mL) to 600 mL distilled water to obtain 620 mL wash solution. Upon dilution, the wash buffer should be stored at 2-8°C where it is stable for at least 3 months.

Assay procedure

(*For a quick guide, refer to Table 1.*)

1. Equilibrate reagents and samples to room temperature before use.
2. Prepare reagents as described in the previous section.
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Label the plate map for duplicates of each standards (S0-S7), and samples (SX).
5. Pipette 25 µL each of standards and samples into the appropriate wells.
6. Pipette 100 µL conjugate into each well.
7. Pipette 100 µL antiserum into each well.
8. Cover the plate by the enclosed foil, place it on the shaker. Incubate the plate for 1 hour, shaking (300 RPM) at room temperature (18-25 °C).
9. Remove the cover and pour the liquid directly over the lab sink. Holding in the upside down position place the plate immediately on an absorbent tissue. *Pay special attention to crossing-over between wells due to droplets backflow!*

10. Add 300 µL wash buffer into each well, decant (tap and blot) or aspirate. Repeat this step 2 times.
    An automatic or manual plate washer can be used. Follow manufacturer's instruction for proper usage.
11. Pour the substrate into a plastic tray and pipette 200 µL to each well. Place it into the dark for 30 minutes. *(If less than the whole volume is used in one assay, do not pipette directly from the bottle, and never fill the unused reagent back into its original bottle).*
12. Pipette 50 µL stop reagent into each well, and shake the plate gently for a few seconds.
13. Measurement in the ELISA photometer at 450 nm wavelength.
14. Calculate the concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	Standard	Sample
Standard	**25**	
Sample		**25**
Conjugate	**100**	**100**
Antiserum	**100**	**100**
Shake for 1 hour at room temperature		
Decant the fluid and blot on filter paper		
Wash buffer	**300**	**300**
Decant the fluid and blot on filter paper		
Repeat the washing step 2 times		
Substrate	**200**	**200**
30 minutes at room temperature in the dark		
Stop reagent	**50**	**50**
Measurement		
Calculate the results		

Calculation of results

The calculation is illustrated using representative data. Data obtained should be similar to those shown in Table 2.

Manual calculation

Calculate the average A.U. for each pair of duplicates. Using semi-logarithmic graph paper plot A.U. for each standard versus the corresponding concentration of rat, mouse corticosterone. Obtain sample values by interpolation of sample A.U. on the standard curve.

Data evaluation using normalized binding

For computerised calculations and/or quality assessment normalised specific binding values, rather than A.U. values are used. Specific binding values can be calculated for each standard and sample according to the following equation:

$$B/B_0\,(\%) = \frac{S1\text{-}7 \,/\, SX\ (AU)}{S0\ (AU)} \times 100$$

S0 is the zero standard, SX is the sample.

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB.

Smoothed spline curve fit is recommended.

Table 2. Typical assay data at 450 nm

Wells	Conc. ng/mL	A.U. 450	A.U. mean	B/B_0 %
S0	0	2.751 2.716	2.734	
S1	1.5	2.279 2.391	2.335	85.4
S2	5	1.786 1.816	1.801	65.9
S3	20	1.094 1.076	1.085	39.7
S4	80	0.546 0.542	0.544	19.9
S5	200	0.293 0.295	0.294	10.8
S6	400	0.168 0.174	0.171	6.3
S7	800	0.095 0099	0.097	3.6

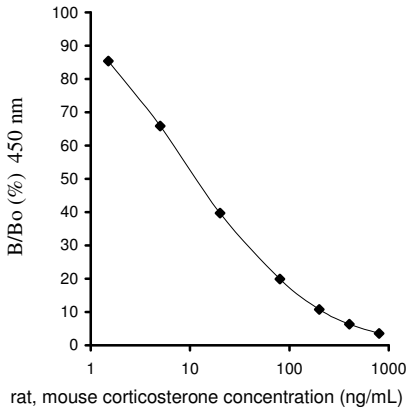

Figure1: A typical standard curve at 450 nm
(*Do not use to calculate unknown samples!)*

Characterization of assay

Sensitivity

For the analytical sensitivity 0.5 ng/mL has been obtained by assaying 16 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean A.U. and its double standard deviation.

Specificity

The cross-reactivity, as determined by the concentration giving 50% B/B_0 with a number of related compounds is shown below:

Compound	Cross-reactivity, %
Rat corticosterone	100
11-Deoxycortisol	<0.039
21-Deoxycortisol	0.078
Cortisol	0.016
11-Deoxycorticosterone	1.645
Progesterone	0.055
17-Hydroxyprogesterone	0.13

Precision

3 samples were assayed in 10 replicates to determine intra-assay precision. Values obtained (ng/mL) are shown below.

Sample	Number of replicates	Mean value	CV%
1	10	12.6	6.52
2	10	119.6	3.06
3	10	211.5	3.69

Reproducibility

To determine inter-assay precision 3 samples were measured in duplicates in 10 independent assays. Values obtained (ng/mL) are shown below.

Sample	Number of runs	Mean value	CV%
1	10	12.9	6.46
2	10	121.1	6.73
3	10	222.5	4.34

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of corticosterone. 97-119 % was obtained for 3 samples.

Added conc. (ng/mL)	Expected conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
0	-	10.6	-
200	105.3	125.3	119
400	205.3	221.3	108
800	405.3	414.2	102
0	-	30.5	-
200	115.3	112.1	97
400	215.3	216.9	101
800	415.3	422.6	102
0	-	191.3	-
200	195.7	194.6	99
400	295.7	287.3	97
800	495.7	503.2	102

Dilution test (linearity)

A serial dilution (1:2 – 1:4) of 3 individual samples was carried out with the zero-standard. The recovery was: 101 -113%.

Samp. No.	Dil. factor	Expected (ng/mL)	Observed (ng/mL)	Reco-very(%)
1	1	-	11.69	-
1	2	5.85	6.18	106
1	4	2.92	3.31	113
2	1	-	221.7	-
2	2	110.85	111.6	101
2	4	55.43	57.3	103

3	1	-	501.5	-
3	2	250.75	284.13	113
3	4	125.38	137.7	110

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions

Chemical hazard

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Caution! The stop reagent is corrosive. Avoid contact with it because it may cause skin irritations and burns.


Use by

LOT Batch code


Caution, consult accompanying documents


Biological risk


Consult operating instructions


Manufacturer

REF Catalogue number

Temperature limitation

Store between 2-8°C

CAL Standard

MP Coated plate

CONJ Conjugate

WASHB Wash buffer

SUB Substrate

STOP Stop reagent

AS Antiserum

Website: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

IZOTOP

INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: (36-1)392-2577, Fax: (36-1)395-9247