

rCorticosterone [¹²⁵I] RIA KIT

(REF: RK-548CT)

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

The [¹²⁵I]rCorticosterone RIA system provides direct quantitative *in vitro* determination of rat Corticosterone in plasma, tissue and cell culture. Rat Corticosterone can be measured in the range 1.56 - 400 ng/mL. Each kit contains materials sufficient for 100 determinations permitting the construction of one standard curve and the assay of 39 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 protein metabolism 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 steroid-receptor 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 ¹²⁵I-labelled corticosterone for a limited number of binding sites on a corticosterone specific antibody.

With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. During incubation the antigen-antibody immunocomplex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter. By plotting binding values against a series of standards containing known amount of corticosterone, a calibration curve is constructed, from which the unknown concentration of corticosterone in samples can be determined. The standard curve and samples should be prepared simultaneously.

Contents of the kit

- 1 vial TRACER, lyophilized, reconstitution with 12.5 mL assay buffer, containing ~ 96 kBq, 2.6 µCi [¹²⁵I]corticosterone. The final solution contains [¹²⁵I]corticosterone in 0.02M borate buffer pH7.4 containing 0.1% (w/v) sodium azide. Store at 2-8 °C.
- 1 vial STANDARD, lyophilized, reconstitution with 2.0 mL assay buffer. The final solution contains corticosterone at a concentration of 400 ng/mL. Store at 2-8 °C.
- 1 vial ANTISERUM (11 mL), ready to use containing rabbit anti-corticosterone serum in buffer pH7.4 with 0.1% (w/v) sodium azide. Store at 2-8 °C.
- 1 vial ASSAY BUFFER concentrate (10.0 mL), dilution to 100 mL. On dilution this will give 0.02 M borate buffer, pH7.4, containing 0.1% (w/v) sodium azide. Store at 2-8 °C.
- 2 boxes COATED TUBE, ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

Pack leaflet

Materials, tools and equipment required

Pipettes or pipetting equipment with disposable tips (25 µL, 100 µL, 500 µL, 2.0 mL and 12.5 mL); disposable polypropylene or polystyrene tubes (12 x 75 mm); distilled water; glass measuring cylinder (100 mL); test tube rack; vortex mixer; plastic foil; adsorbent tissue; gamma counter.

Note: For Option A (the shorter incubation protocol), the following additional equipment will be required: shaker.

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 heparin or 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. **Prior to assay, corticosterone needs to be displaced from cortisol binding globulin (CBG) in plasma/serum. We recommend heating samples to 60°C for 30 minutes, for example in a water bath, then cool to ambient temperature and assay.**

Samples may need to be diluted due to the high rat corticosterone plasma/serum concentration. We recommend dilutions of 1:10 or greater depending on expected concentration, for example dilute 50 µL sample with 450 µL buffer. Dilution of samples can be done before heating to inactivate CBG.

Tissue samples

Adrenal samples should be frozen immediately after removal and stored at -80°C until required. Prior to analysis, glands should be homogenized or sonicated in appropriate buffer. Buffers described in the literature include Krebs-Ringer bicarbonate buffer (KRB) containing glucose. Samples may need to be diluted depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

Cell culture

Cell culture media should be collected and stored below -15°C prior to analysis. Samples may need to be diluted depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

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 and on the quality certificate.

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

Assay buffer: Transfer the contents of the bottle to a 100 mL measuring cylinder and dilute to 100 mL with distilled or deionized water. Mix well. Assay buffer is used to redissolve the standard and the tracer.

Tracer and standard: Carefully add the required volume of assay buffer and replace stopper. Mix the contents of the bottles by inversion and swirling, taking care to avoid foaming.

Preparation of working standards

- Label 8 polystyrene or polypropylene tubes 1.56, 3.13, 6.25, 12.5, 25, 50, 100 and 200.
- Pipette 500 µL of assay buffer into all tubes.
- Into the 200 tube pipette 500 µL of stock standard (400 ng/mL) and vortex thoroughly.
- Transfer 500 µL from the 200 tube to the 100 tube and vortex thoroughly.
- Repeat this doubling dilution successively with the remaining tubes.
- 25 µL aliquots from each serial dilution give rise to 8 standard levels of corticosterone ranging from 1.56 to 200 ng/mL.

Note: Twenty five microlitres (25 µL) of the reconstituted stock standard provided serves as the top standard (400 ng/mL). Working standards should be freshly prepared before each assay, and not re-used.

Assay procedure

Option A

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

1. Equilibrate all reagents to room temperature.
2. Prepare reagents and assay standards as described in the previous section.
3. Label 12x75 mm **disposable tubes** in duplicate for total count (TC), **coated tubes** for non-specific binding (NSB), zero standard (Bo), standards and samples.
4. Pipette **125 µL** assay buffer into NSB tubes and **25 µL** assay buffer into Bo tubes.
5. Starting with the most dilute, pipette **25 µL** of each standard (S1-9) into the appropriately labelled tubes.
6. Pipette **25 µL** unknown sample (SX) directly into appropriately labelled tubes.
7. Pipette **100 µL** of tracer into all tubes. The TC tubes should be stoppered and put aside for counting.
8. Pipette **100 µL** antiserum into all tubes except NSB and TC.
9. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
10. Incubate tubes for 1 hour, **shaking** at room temperature.
11. Add 2.0 mL distilled water to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
12. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.
13. 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	TC	NSB	Bo	Stan - dard	Sam- ple
Buffer	-	125	25	-	-
Stan- dard	-	-	-	25	-
Sample	-	-	-	-	25
Tracer	100	100	100	100	100
Anti- serum	-	-	100	100	100
Shake for 1 hour at room temperature					
Distilled water	-	2000	2000	2000	2000
Decant the fluid and blot on filter paper					
Count radioactivity (60 sec/tube).					
Calculate the results.					

Table 2. Typical assay data

Tube	Conc. ng/mL	Mean counts (cpm)	B/TC (%)	B/Bo (%)
TC	-	29246	-	-
NSB	-	449	1.5	-
Bo	-	18578	62.0	-
S1	1.56	17474	-	93.9
S2	3.13	16577	-	88.9
S3	6.25	15020	-	80.4
S4	12.5	12807	-	68.2
S5	25	10221	-	53.9
S6	50	7484	-	38.8
S7	100	5158	-	25.9
S8	200	3410	-	16.3
S9	400	2268	-	10.0

Note: The counts were obtained using new tracer. The counts will decline in line with the age of the tracer.

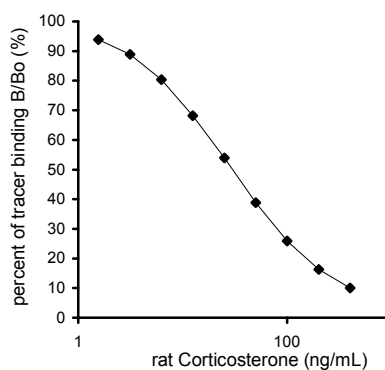


Figure 1: A typical standard curve (Do not use to calculate unknown samples!)

Option B

(For a quick guide, refer to Table 3.)

1. Equilibrate all reagents to room temperature.
2. Prepare reagents and assay standards as described in the previous section.
3. Label 12x75 mm **disposable tubes** in duplicate for total count (TC), **coated tubes** for non-specific binding (NSB), zero standard (Bo), standards and samples.
4. Pipette **125 µL** assay buffer into NSB tubes and **25 µL** assay buffer into Bo tubes.
5. Starting with the most dilute, pipette **25 µL** of each standard (S1-9) into the appropriately labelled tubes.
6. Pipette **25 µL** unknown sample (SX) directly into appropriately labelled tubes.
7. Pipette **100 µL** of tracer into all tubes. The TC tubes should be stoppered and put aside for counting.
8. Pipette **100 µL** antiserum into all tubes except NSB and TC.
9. Vortex mix all tubes thoroughly. Seal all tubes with a plastic foil and incubate tubes for 2 hours at room temperature (15-30 °C).
10. Add 2.0 mL distilled water to each tube. Decant the supernatant from all tubes by

the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.

11. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter
13. Calculate the concentrations of the samples as described in calculation of results or use special software.

Table 3. Assay Protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	TC	NSB	Bo	Stan - dard	Sam- ple
Buffer	-	125	25	-	-
Stan- dard	-	-	-	25	-
Sample	-	-	-	-	25
Tracer	100	100	100	100	100
Anti- serum	-	-	100	100	100
Vortex mix and incubate for 2 hours at room temperature					
Distilled water	-	2000	2000	2000	2000
Decant the fluid and blot on filter paper					
Count radioactivity (60 sec/tube).					
Calculate the results.					

Table 4. Typical assay data

Tube	Conc. ng/mL	Mean counts (cpm)	B/TC (%)	B/Bo (%)
TC	-	29298	-	-
NSB	-	657	2.2	-
Bo	-	12398	40.1	-
S1	1.56	12162	-	97.9
S2	3.13	11463	-	92.0
S3	6.25	10587	-	84.6
S4	12.5	9135	-	72.2
S5	25	7503	-	58.3
S6	50	5624	-	42.3
S7	100	3905	-	27.7
S8	200	2680	-	17.2
S9	400	1937	-	10.9

Note: The counts were obtained using new tracer. The counts will decline in line with the age of the tracer.

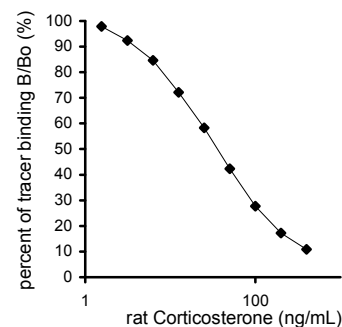


Figure 2: A typical standard curve (Do not use to calculate unknown samples!)

Calculation of results

Calculate the average count per minute (cpm) for each pair of assay tubes.

Calculate the percent NSB/TC using the following equation:

$$\text{NSB/TC (\%)} = \frac{\text{NSB (cpm)}}{\text{TC (cpm)}} \times 100$$

If the counter background is high, it should be subtracted from all counts.

Users may wish to subtract the average NSB cpm from all tubes except TC. If so the appropriate correction should be made. Calculate the percent Bo/TC using the following equation:

$$\text{Bo/TC (\%)} = \frac{\text{Bo (cpm)} - \text{NSB (cpm)}}{\text{TC (cpm)}} \times 100$$

Calculate the percent bound for each standard and sample using the following equation:

$$\text{B/Bo(\%)} = \frac{\text{S1-9/SX (cpm)} - \text{NSB (cpm)}}{\text{Bo (cpm)} - \text{NSB (cpm)}} \times 100$$

A standard curve can be generated by plotting the percent B/Bo as a function of the log corticosterone concentration.

Plot B/Bo(%) (y-axis) against concentration ng standard per mL (x-axis). The concentration (ng/mL) value of the samples can be read directly from the graph (see Figure 1 and 2).

Characterization of assay

Stability

The components of this assay system will have a shelf-life of at least 4 weeks from the date of despatch.

Upon arrival, all components should be stored at 2-8 C where they are stable until the expiry date printed on the end pack label. Once reconstituted, all reagents should be stored at 2-8 C where they are stable for at least 28 days.

Sensitivity

The sensitivity, defined as the amount of corticosterone needed to reduce zero dose binding by two standard deviations was 0.4 ng/mL.

Specificity

The cross-reactivity, as determined by the concentration giving 50% B/Bo 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

The within-assay precision for duplicate determinations was calculated by repeatedly

measuring an unknown in the assay. The result obtained was 134 ng, CV=1.7%. The between assay precision was assessed by repeated measurement of the same samples in successive assays using different batches of reagents and different operators. These results are shown below:

Sample	(n)	Mean	CV %
1	10	25.72	4.1
2	10	67.72	5.6
3	10	198.6	3.5

Assay Parallelism

The following correlation was obtained between sample dilution and measured concentration in unextracted plasma and serum.

Sample dilution	plasma	
	Measured conc. (ng/mL)	Calculated conc. (ng/mL)
1:5	88.8	444
1:10	47.6	476
1:20	26.0	520
Sample dilution	serum	
	Measured conc. (ng/mL)	Calculated conc. (ng/mL)
1:5	112.4	562
1:10	56.4	564
1:20	23.6	472

Recovery

The following recovery was obtained when corticosterone was added to rat plasma and serum. Corticosterone was added at ~80 ng/mL at each dilution and endogenous levels accounted for in calculation of recovery.

Sample dilution	plasma		
	Expected conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
1:5	145.6	164.8	113.2
1:10	112.8	130.4	115.6
1:20	96.4	109.6	113.7
Sample dilution	serum		
	Expected conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
1:5	147.6	160.0	108.4
1:10	114.0	145.6	127.7
1:20	96.8	108.8	112.4

Additional information

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

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 112.5 mg.

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).

Safety data sheet

Product name:

Sodium azide

CAS No. 26628-22-8

R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas.

S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Composition:

Sodium azide solution.

Hazards identification:

Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.

First aid measures:

In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.

Fire fighting measures:

Dry chemical powder. Do not use water.

Accidental release:

Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.

Handling and storage:

Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.

Personal protection:

See above instructions for handling and storage.

Physical and chemical properties:

Formula weight: 65.01. Density: 1.850.

Stability and reactivity:

Avoid contact with metals and acid chlorides. This yields a very toxic gas.

Toxicological information:

LD50: 27 mg/kg oral, rat

LD50: 20 mg/kg skin, rabbit

Ecological information:

Not applicable

Disposal consideration:

Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.








Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.

Transport information :

No special considerations applicable.

Regulatory information:

The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

	Use by	AS	Antiserum
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	BUF	Assay buffer
	Manufacturer		Temperature limitation Store between 2-8 °C
REF	Catalogue number		Radioactive Material

Website: <http://www.izotop.hu>

Technical e-mail: immuno@izotop.hu

Commercial e-mail: commerce@izotop.hu



INSTITUTE OF ISOTOPES CO. LTD.

1535 Budapest. Pf.: 851.

Tel.: (36-1)392-2577, Fax: (36-1)395-9247