CORTISOL [^{125}I] RIA KIT
(Ref: **RK-240CT)**

Description

The Cortisol [^{125}I] assay system allows the quantitative in vitro determination of cortisol in human serum. Cortisol can be assayed in the range of 0-1600 nmol/l (0-580 ng/ml). Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and assay of 42 unknowns and 1 control in duplicate.

Introduction

Cortisol is the main glucocorticoid which is produced by the adrenal cortex. It regulates carbohydrate, protein, fat and purine metabolism, electrolyte and water balance. Cortisol has a role in blood pressure regulation and resolution of inflammation.

The determination of cortisol levels can be used for diagnosing the functional disturbances of the hypothalamus-pituitary-adrenal cortex (HPA) axis.

In normal individuals cortisol secretion has a characteristic rhythm: the cortisol level is the highest in the morning, by the evening it decreases to approximately half that level.

Principle of the method

This assay is based on the competition between unlabelled cortisol and fixed quantity of ^{125}I-labelled cortisol for limited number of binding sites on cortisol specific antibody. Allowing to react a fixed amount of tracer and antibody with different amounts of unlabelled ligand, the amount of tracer bound by the antibody will be inversely proportional to the concentration of unlabelled ligand.

During a 2-hour incubation period with continuous agitation, immuno-complex is immobilized on the reactive surface of test tubes. After incubation the reaction mixture is discarded, and the radioactivity is measured in a gamma counter.

The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against a series of calibrators containing known amounts of cortisol, a calibration curve is constructed, from which the unknown concentration of cortisol in patient samples can determined.

Contents of the kit

1 vial	TRACER, Ready to use. 55 ml per vial, containing < 260 kBq ^{125}I-Cortisol in buffer with 0.1% NaN_3
6 vials	STANDARDS, Ready to use. 0.5 ml per vial, containing 0, 40, 100, 250, 650, 1600 nmol/l Cortisol in serum with 0.1% NaN_3
1 vial	ANTISERUM, Ready to use. 55 ml per vial, containing polyclonal anti-Cortisol (rabbit) IgG in buffer with 0.1 % NaN_3
1 vial	CONTROL SERUM. Lyophilised human serum with 0.1% NaN_3
2 boxes	COATED TUBE, 2x50 pcs, 12x75 mm packed in plastic boxes.
	Quality certificate.
	Pack leaflet.

Materials and equipment required

Test tube rack, precision pipettes with disposable tips (10 and 500 µl), vortex mixer, shaker, plastic foil, absorbent tissue
Gamma counter
Recommended tools and equipment
repeating pipettes

Preparation of reagents

Add 500 µl distilled water to the lyophilised control serum. Mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes.

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Sera can be stored at 2-8 °C for two days after collection. For later analysis they should be stored deep-frozen.

Assay procedure

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

1. Equilibrate reagents and samples to room temperature before use (min. for an hour).
2. Label coated tubes in duplicate for each standard (S1-S6), control serum (C) and samples (Sx). Optionally, label two uncoated test tubes for total count (T).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 10 µl each of standards, control and samples into the properly labelled tubes.
5. Pipette 500 µl of tracer into each tube.
6. Pipette 500 µl antiserum into each tube except T.
7. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube
8. Incubate tubes for 2 hours at room temperature.
9. Aspirate or 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.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the cortisol concentrations of the samples as described in calculation of results.

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

Tubes	T	S_{1-6}	C	S_X
Standard		10		
Control			10	
Sample				10
Tracer	500	500	500	500
Antiserum		500	500	500
Shake for 2 hours at room temperature				
Decant the fluid and blot on filter paper				
Count all tubes				

Calculation of results

The assay data should be similar to those shown in Table 2. Calculate the average counts per minute (CPM) for each pair of assay tubes.

Calculate the percent B_0/T for zero standard (S_1) by using the following equation:

$$B_0/T\ \% = 100 * S_1\text{(cpm)} / T\text{(cpm)}$$

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0\ \% = 100 * S_{2\text{-}6}\ ;\ C\ ;\ S_x\text{(cpm)} / S_1\text{(cpm)}$$

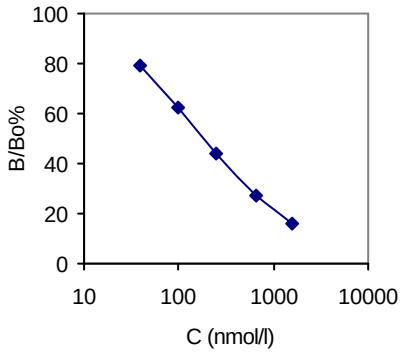
For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 3% of total count.

Using semi-logarithmic graph paper plot B/B_0% for each standard versus the corresponding concentration of cortisol. Figure 1 shows a typical standard curve. Determine the cortisol concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Automated data processing systems are also available.

Table 2. Typical Assay Data

Tubes	Counts CPM1	Counts CPM2	Counts CPM	B/T %	B/B_0 %
T	90172	89562	89867		
S1	51816	51699	51758	57.6	100
S2	41325	40790	41058	45.7	79.3
S3	32114	32332	32223	35.9	62.3
S4	23112	22076	22594	25.1	43.7
S5	14264	13917	14091	15.7	27.2
S6	8272	8347	8309,5	9.2	16.1
C	19600	18893	19247	21.4	37.2

Figure 1.
A typical standard curve
(Do not use to calculate sample values)



Conversion of SI units can be performed according to the following formula:
1 nmol/l = 0.362 ng/ml

Characterization of the assay

Assay parameters

parameters	**value**
B_0/T	56 ± 10 %
ED-50	180±36 nmol/l

Specificity

Cross reactivity of the cortisol antiserum used in the kit with various substances is shown below:

Added steroid concentration	70 nM	700 nM
	Apparent cortisol concentration nM	
Corticosterone	6	30
17-a-hydroxy-progesterone	<DL	13
Cortisone	<DL	6
11-deoxycortisol	10	56
Deoxy-corticosterone	<DL	12
Prednisolone	64	428
Dexamethasone	<DL	15

Steroids shown below up to 700 nM are undetectable in the measurement of cortisol: 17ß-estradiol, progesterone, 5-alfa dihydrotestosterone, 5-ß-dihydro-19-nor-testosterone, testosterone, androstenedion, androstenediol, 17-a-methyl-testosterone, androstanediol, aldosterone, pregnenolone, 19-nor-testosterone, dehydroepiandrosterone, estriol, estrone.

Sensitivity

2,9 nmol/l, defined as the concentration 2 standard deviations from the zero standard.

Precision, reproducibility

Inter-assay (7 assays in 2 rep.)		Intra-assay (1 assay in 10 rep.)	
Mean	CV%	Mean	CV%
45.7	1.65	46.5	6.25
86.0	3.15	84.6	3.70
223.9	3.33	237.7	6.81
339.6	1.77	398.3	3.16
427.1	2.87	439.6	2.47
792.6	4.56	766.5	4.51
1113.9	4.70	1142.8	5.62

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of Cortisol. Recovery for 16 serum samples spiked with Cortisol were:
92 ± 7 %

Expected reference values

It is recommended that each laboratory establish its own reference intervals. The expected values presented here are based on testing of apparently healthy blood donors (90 males and 90 females). **Samples were obtained between 8-11 AM**. From statistical analysis, the following results were obtained:
mean ± SD=353± 139 nmol/l
absolute range= 127-859 nmol/l
Serum cortisol results were normally distributed after log transformation and the 95% confidence interval was calculeted. **Range of normal cortisol values based on 95% interval is: 147-726 nmol/l.**

Additional information

Limitations

The reagents supplied in this kit are optimized to measure cortisol levels in serum and plasma.
Avoid freeing and thawing of reagents and specimens.
Hemolyzed and lipemic specimens may give false values and should be avoided.

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
This kit should only be used for in vitro diagnostic purposes.

Storage

Store the reagents between 2 and 8 °C. At this temperature each reagent is stable until expiry date of the KIT.

Availability

From stock.

Shelf life

The actual expiry date is given on package label and in the quality certificate.

Precautions and warnings

Radioactivity

This kit contains radioactive material. Receipt, acquisition, possession, or use of radioactive materials are subject to regulations, and a licence of (inter)national authorizing bodies. It is the responsibility of the user to ensure that local regulations or codes of practice are satisfied.

Potentially infectious materials

Human blood products provided as components of this product have been obtained from donors tested individually and found negative for Human Immunodeficiency Virus antibody (HIV-Ab) as well as for Hepatitis B surface Antigen (HBsAg) using approved EIA methods.

Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV), or other infectious agents are absent, and all human blood samples should be considered potentially infectious.

Chemical and other hazard

Some components contain sodium azide (0.1 % w/v) as an Antimicrobial Agent. Dispose the waste by flushing it with copious amounts of water to avoid build up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 114 mg.

	Used by	LOT	Batch code
8° 2°	Temperature limitation	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device	AS	Antiserum
	Manufacturer		
REF	Catalogue number		
	Radioactive material		

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