



11-Desoxycortisol RIA

For the quantitative determination of 11-Desoxycortisol in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 38-DESHU-R96
Size: 96 determinations
Version: 090604/1 – ALPCO 1/20/2010

ALPCO Diagnostics

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1. INTENDED USE

The 11-Desoxycortisol (Compound S) is an intermediate steroid in the biosynthesis of glucocorticoids. Precursor of cortisol, it comes from 17-hydroxyprogesterone after action of the 11- β -hydroxylase. This parameter is interesting for the study of surrenal enzymatic deficiency in 11- β -hydroxylase, which is responsible for congenital surrenal hyperplasia in children and hyperandrogenics in adult women.

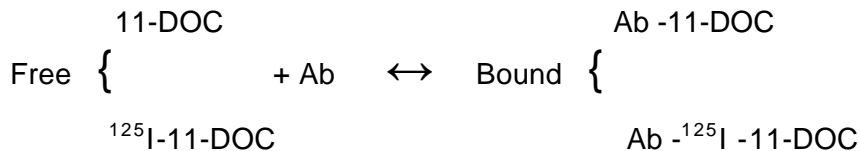
Under hypothalamic-pituitary control via the Adrenocorticotropic hormone (ACTH), the secretion of 11-Desoxycortisol follows a nycthemeral variation: it reaches a maximum in the morning (around 8 a.m.) and a minimum during the night (between 0 to 4 a.m.)

The measure of 11-Desoxycortisol can be performed in serum using immunological competition methods (RIA).

The metopyrone inhibits the 11- β -hydroxylase and the conversion of the 11-Desoxycortisol to cortisol. The metopyrone test is an indicator of the ACTH reserve.

2. PRINCIPLE OF THE METHOD

The 11-Desoxycortisol (DOC) RIA obeys the law of mass action according to the following equation:



Since the concentrations of $^{125}\text{I-11-DOC}$ and coated antibodies are constant, the advancing state of the equation depends on the concentration of 11-DOC. The amount of $^{125}\text{I-11-DOC}$ bound to the coated tube is inversely proportional to the concentration of 11-DOC in the sample.

Following the incubation, the tube is aspirated to remove excess unbound ^{125}I labeled 11-DOC.

Sample concentrations are read from a calibration curve.

3. MATERIALS PROVIDED AND STORAGE

Stored at 2 - 8°C, the materials can be used up to the expiration date printed on each label.

3.1. **2 x 48 Polystyrene tubes** (12 x 75 mm)

coated with anti-11-DOC polyclonal antibodies.

Allow the coated tubes to systematically reach room temperature before use. Store at 2-8°C.

3.2.

Ag	^{125}I
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 $^{125}\text{I-11-DOC E}$ analog tracer (yellow, 52 mL)

1 bottle, containing < 5 μCi (185 kBq) of ^{125}I -labeled 11-DOC analog in a protein-based buffer containing < 0.1% sodium azide as a preservative. Store at 2-8°C.

3.3.

CAL	N
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11-DOC Calibrators

5 vials, 0.5 ml each, containing 0.3, 1.5, 5, 20, and 65 ng/ml 11-DOC calibrators in human serum with < 0.1% sodium azide as a preservative. Store at 2-8°C. Can be used up to the expiration date printed on each label.

3.4.

CAL 0

11-DOC Zero Calibrator

One vial, 1 ml, containing 0 ng/ml 11-DOC zero calibrator in human serum with < 0.1% sodium azide as a preservative. Store at 2-8°C. Can be used up to the expiration date printed on the label.

3.5.

CONTROL N

Control 1 – 2

2 vials, lyophilized, 0.5 ml each, levels I and level II containing low and high 11-DOC levels in human and horse serum with < 0.1% sodium azide as a preservative.

Store at 2-8°C. Can be used up to the expiration date printed on each label.

Before use, reconstitute the contents of the controls with 0.5 ml of distilled water.

3.6.

WASH SOLN CONC

Concentrated Wash Buffer

1 bottle concentrated buffered solution containing sodium azide (NaN_3 < 0.1 %). Pour the solution into 700 ml of distilled water.

Note: Conversion factor: 1 ng/ml = 2.886 nmol/L

$$1 \text{ nmol/L} = 0.3465 \text{ ng/ml}$$

4. MATERIALS REQUIRED BUT NOT PROVIDED

- bench surfaces protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers appropriately labeled and designed as suitable for solid or liquid radioactive materials.
- manual or automated precision micropipettes for dispensing samples or reagents without cross-contamination.
- absorbent paper.
- vacuum pump connected through a trap for aspiration.
- water bath.
- a gamma scintillation counter.
- appropriate graph paper or computer program for plotting the results.

5. METHODOLOGY

5.1. Collection and handling of blood samples:

The blood sample may be collected into a dry tube.

After separation from the red blood cells, serum samples may be assayed immediately, within 24 hours if stored at 2 - 8°C, or after periods of up to several months if stored at -20°C. Repeated freezing and thawing must be avoided.

If the samples are from subjects younger than 2 years old, the 11-Desoxycortisol must be assayed immediately after an extraction step. Please contact ALPCO to receive this extraction procedure:

(800) 592-5726

www.alpco.com

5.2. Assay procedure:

Reagents stored at 2 - 8°C must be brought to room temperature prior to use. Do not mix reagents of different lots. Label the tubes for T (« Total Count », do not use coated tubes), calibrators, samples, and control sera.

Perform the assay in duplicate. Calibrators, controls, and samples must be assayed at the same time.

1. Calibration curve:

Pipette 25 µl of each calibrator into the corresponding tubes.

2. Unknowns and control sera:

Pipette 25 µl of each sample or control sera into the corresponding tubes.

3. Add 500 µl of ¹²⁵I -11-DOC tracer to each tube.

4. Vortex, cover, and incubate 2.5 hours at 37 ± 2°C.

5. Add 2 ml of washing solution to each tube, *except total count tubes*, then aspirate or decant carefully.

6. Repeat step 5.

7. Count the radioactivity fixed in each tube for at least 60 seconds.

Data processing :

Determine the mean count rate for each set of duplicate tubes. Calculate the ratio B/B₀ as follows:

$$B/B_0 \% = [\overline{\text{CAL or Sample cpm}} / \overline{B_0 (\text{CAL } 0) \text{ cpm}}] \times 100$$

Draw the calibration curve on semilogarithmic paper by plotting the ratio B/B₀ % (linear scale) obtained for each calibrator versus its respective concentration expressed in ng/ml (logarithmic scale). 11-DOC concentrations in samples may be read directly from the calibration curve.

If a computer is used to calculate the results, the data can be fitted to the appropriate equation: spline smoothed.

5.3. Example of a typical assay:

	Content (ng/ml)	cpm 1st duplicate	cpm 2nd duplicate	Mean cpm	B/B ₀ (%)	11-DOC (ng/ml)
Total counts	-	63015	62510	62762	-	-
CAL 0	0	31865	31445	31655	100	-
CAL 1	0.3	27429	28004	27711	87.6	-
CAL 2	1.5	20079	19803	19941	62.9	-
CAL 3	5	13246	13152	13199	41.7	-
CAL 4	20	6369	6509	6439	20.3	-
CAL 5	65	3464	3480	3472	11	-
C1 Low	-	22977	22569	22773	72	1
C2 High	-	13711	13699	13705	43.3	4.5
Sample	-	4172	4242	4214	13.3	45.2

Example of a typical assay, do not use for calculations

6. PERFORMANCE CHARACTERISTICS

6.1. Specificity:

Steroid	% Cross-reactivity
11-Desoxycortisol	100
17 α -Hydroxyprogesterone	5.6
Desoxycorticosterone	0.46
Progesterone	0.59
Cortisol	0.09
Estradiol-17 β	0.03
Androstenedione, Corticosterone, Dexamethasone, Cortisone, Testosterone, DHEA-S, Aldosterone	N.D.

6.2. Minimum detectable concentration of 11-DOC:

The minimum detectable concentration has been assayed at 0.11 ng/ml and corresponds to the concentration given by two standards deviations below the mean cpm of 20 replicate determinations of the zero calibrator.

6.3. Recovery test:

When sera of known 11-DOC content have their 11-DOC supplemented by the addition of 11-DOC in equal volumes (1/1), a satisfactory correlation between theoretical and assayed 11-DOC is obtained.

Added 11-DOC (ng/ml) (1:1 in serum sample)	1.5	5	20	65
Theoretical (ng/ml)	0.85	2.6	10.1	32.6
Assayed 11-DOC (ng/ml)	0.70	2.5	10.2	30.2
% Recovery	82.4	96.2	101	92.6

6.4. Dilution test:

The dilution test indicates that there is immunological identity between the 11-DOC present in the sample and the 11-DOC used to calibrate the test.

Dilution Factor	1	1/2	1/4	1/8	1/16	1/32	1/64
Assayed 11-DOC (ng/ml)	40.1	18.4	9.8	4.7	2.5	1.1	0.6
Expected 11-DOC (ng/ml)	-	20.05	10	5	2.5	1.25	0.63
% Recovery	-	92	98	94	100	88	96

6.5. Reproducibility:

	Within assay variation 5 replicates		Between assay variation 8 replicates	
	Mean (ng/ml)	CV%	Mean (ng/ml)	CV%
Pool 1	1.1	6.2	1.95	8.7
Pool 2	3.7	5.2	5.48	11.5
Pool 3	28.3	7.7	36.85	15.1

7. LIMITATIONS OF THE PROCEDURE

- This kit is for research use only. It is not for use in diagnostic procedures.
- Do not use lipemic, hemolyzed, icteric, or turbid specimens.

8. EXPECTED VALUES

Without Stimulation < 7.2 ng/ml

After Stimulation with Metopyrone 72 – 225 ng/ml

9. WARNING AND PRECAUTION

For research use only

Safety

This kit contains ¹²⁵I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiation.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use, and exchange of radioactive products are subject to the legislation of the end user's country. In no case should the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned up immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, and anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS, or other infections. Therefore, reagents and serum/plasma specimens should be handled as if potentially infectious. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat, or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.