CORTISOL

Enzyme-immunoassay for the quantitative determination of Cortisol in human serum

REF **GD7250 00**

CE

IVD

INDICATION

Cortisol is a steroid hormone released from the adrenal cortex in response to ACTH hormone (produced by the pituitary gland). Cortisol acts through specific intracellular receptors and has effects in numerous physiologic systems, including immune function, glucose-counter regulation, vascular tone, and bone metabolism. Cortisol is excreted primarily in urine in an unbound (free) form.
Cortisol is bound, in plasma, from corticosteroid-binding globulin (CBG, transcotin), with high affinity, and from albumin. Only free cortisol is available to most receptors.
The amount of cortisol present in the serum undergoes diurnal variation, with the highest levels present in the early morning, and lower levels in the evening, several hours after the onset of sleep. Cortisol is involved in the response of chronic stress. Prolonged cortisol secretion causes muscle wastage, hyperglycaemia, and suppresses immune/inflammatory responses. The same consequences arise from a long-term use of glucocorticoid drugs.

PRINCIPLE OF THE ASSAY

This test is based on "one step" competition enzyme immunoassay principle (ELISA). Tested specimen is placed into the microwells coated by specific anti-Cortisol antibodies simultaneously with Cortisol conjugated to Horseradish peroxidase (HRP). Cortisol from the specimen competes with the conjugated antigen for coated antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate solution. The developed colour, detected at 450 nm, is inversely related to the quantity of Cortisol present in the specimen.
Cortisol concentration in the sample is calculated based on a series of standards.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with anti-Cortisol monoclonal antibodies. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B – Enzymatic Tracer**
   1 vial of 12 ml.
   Ready to use solution containing Cortisol, conjugated with Horseradish peroxidase (HRP) in a proteic stabilized matrix with 0.1% ProClin 300 as preservative.

3. **Reagent C – Washing Solution 25x**
   1 vial of 50 ml.
   Concentrated solution to be diluted 1:25 with distilled water. It contains a detergent in Phosphate buffer.

4. **Reagent D/E – Chromogen/Substrate**
   1 vial of 12 ml.
   Ready to use solution containing Tetramethylbenzidine (TMB) and H_2O_2 in Citric acid buffer.
   Avoid any skin contact and light exposure.

5. **Reagent F – Stop Solution**
   1 vial of 15 ml.
   Ready to use solution containing Sulphuric acid 0.2 M.
   Avoid any skin contact.

6. **Cortisol Standards:**
   6 vials of 0.5 ml each.
   Ready to use human serum based liquids containing Cortisol and 0.1% ProClin 300 as preservative.
   Approximately Cortisol concentrations are the following:
   S_0: 0 nmol/l, **S_1**: 15 nmol/l, **S_2**: 90 nmol/l,
   S_3: 400 nmol/l, **S_4**: 800 nmol/l, **S_5**: 1800 nmol/l.
   For SI units: nmol/l x 0.362 = ng/ml.
   Actual concentrations to be used for calculation are stated on the labels of the vials.

7. **Cortisol Control:**
   1 vial of 0.5 ml.
   Ready to use liquid containing human serum with a defined quantity of Cortisol and 0.1% Proclin 300, as preservative.
   Refer to the vial label for acceptable range.

8. **Cardboard sealers**
   2 cardboard sealers to be used to cover the plate during the incubations.

9. **Package insert:** instruction for use GD7250 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Chromogen/Substate and Stop Solutions.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 25, 100 and 150 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Thermoshaker at 37 (± 0.5) °C
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Serum only may be used. The kit is not calibrated for the determination of Cortisol in plasma, saliva or other specimens of human or animal origin. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier. Separate serum as soon as possible to avoid any hemolysis. Samples can be stored at 2–8 °C for a short time (max three days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing. Highly lipemic, hemolysed, preserved by sodium azide or microbiologically contaminated samples should not be used in the assay.

REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:25 with distilled or ELISA grade water (e.g.: 20 ml of Reagent C + 480 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 3 days at 2-8 °C. The concentrated solution may present a sediment that can be dissolved at 35-39 °C and shaking. It is recommended to store washing solution at room temperature for immediate use.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.
We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.
Generally, 5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.
In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of 20 minutes. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.

ASSAY SCHEME

1. Put the desired number of microstrips into the frame.
2. Follow the scheme:

		Microplate wells coated with anti-Cortisol antibody	
	REAGENTS	**Standards, Control**	**Sample**
Immunological reaction	Standards, Control	25 µl	-
	Sample	-	25 µl
	Reagent B (Enzymatic Tracer)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate on a **thermoshaker** (approximately 500-800 rpm) **30 minutes at 37 (± 0.5) °C***		
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid		
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **20-30 minutes at room temperature** (22-28 °C), avoiding light exposure		
	Reagent F (Stop Solution)	150 µl	150 µl
	- Gently mix for 5-10 seconds - Read the absorbance of each well at 450 nm.		

* Alternatively, incubate for 60 minutes at 37 (± 0.5) °C

CALCULATION OF RESULTS

1. Calculate the mean of the absorbance values for each point of the standard curve, control and of each sample.
2. Draw a calibration curve on a linear graph paper with the mean optical densities on the Y-axis and the standards concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Interpolate the values of the samples on the standard curve to obtain the corresponding values of concentration expressed in nmol/l.

VALIDITY OF THE TEST

For the test to be valid the following criteria must be met:

1. Standard 0 nmol/l OD 450 nm: ≥ 1.3
2. Calculated value of Control should be within the established range stated on the label.

EXPECTED VALUES

From data obtained by Minias Globe Diagnostics the following reference range is suggested. Otherwise, it is recommended that each laboratory establishes its own normal and abnormal values according to the examined population.

Subjects	Range (nmol/l)
Male/Female:	63-600

Note:

- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory results is only a part of the total clinical picture of the patient.
- Serum Cortisol values may be depended from administration of prednizolone, prednisone and other structurally related corticosteroids.

ANALYTICAL PERFORMANCES

Assay range

The range of the assay is 0 – 1800 nmol/l

Analytical Sensitivity

The lower detection limit is 5 nmol/l. The sensitivity was calculated by determining the variability of Standard 0 nmol/l and using the 2 SD (95% certainty) statistics.

Precision

a. Intra Assay Variation

Sample	Mean, nmol/l	SD	%CV
1	359	8.62	2.4
2	511	7.15	1.4

b. Inter Assay Variation

Sample	Mean, nmol/l	SD	%CV
1	361	18.77	5.2
2	514	24.67	4.8

Specificity

The following compounds were tested for cross-reactivity:

Substance	Cross reactivity, %
Cortisol	100
Cortisone	0.47
Corticosterone	10.70
Testosterone	0.27
Estradiol	< 0.001
Estriol	< 0.001
Androstenedione	0.10
Progesterone	4.60

Recovery
Spiked samples were prepared by adding defined amounts of Cortisol to patient serum sample. The results are tabulated below:

Added (nmol/l)	Measured (nmol/l)	Expected (nmol/l)	Recovery (%)
-	18.2	18.2	-
1800	887.0	909.1	97.5
800	402.0	409.1	98.2
0	8.2	9.1	90.1

Accuracy
The present kit was compared with a Chemiluminescent microparticle immunoassay as a reference test. 60 specimens were tested (values ranged from 89 to 946 nmol/l).
The following linear regression curve was calculated:

y = 0.92x – 4.18 nmol/l r = 0.96

PRECAUTIONS IN USE
The reagents are not considered harmful according to the 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

REFERENCES
1. Peters J.R., et al., Clin Edocrinol. 17:583 (1982).
2. Papanicolaou,D.A. et al J. Cli Endocrinol Metab 87(10): 4515-4521.
3. Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol. Obstet. Invest. 40:139-140 (1995).

Manufacturer:
MINIAS GLOBE DIAGNOSTICS SRL
Via Galileo Galilei 38 - Seggiano di Pioltello (Milan) Italy
Tel: +39 02 929189 1 - Fax:+39 02 929189 39

Met. GD725000.0 ing
Edition: 2010/05/20