Human Cortisol ELISA Kit

to quantitatively determine Cortisol in Human Blood Serum or Plasma

INSTRUCTION MANUAL

FOR ELISA KIT No: LT39910EAYQ

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INTENDED USE

This kit is used to quantitatively determine the Human Cortisol in the sample of Human Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X2

BACKGROUND

Cortisol is a glucocorticoid with a MW of 362.5 Dalton. Cortisol is the major hormone secreted by adrenals. In blood, cortisol is found mostly in a bound form, transcortin being the carrier. Cortisol secretion undergoes circadian rhythms with maximal (up to 700 nmol/l) concentrations seen in the morning (6–9 am) and minimal (up to 55 nmol/l) – in the midnight. During pregnancy, Cortisol blood level is continuously increasing by up to 5-fold of initial concentration before delivery, its circadian rhythm being altered. Cortisol plays an important role in development of alveolar epithelium and surfactant secretion, this being of major importance for the first inhale of a newborn. Elevated Cortisol concentrations in blood are found in secreting tumours of adrenals, in virilizing hyperplasia of adrenals, in Cushing syndrome, in ACTH-producing tumours, during surgical stress, in cardiac insufficiency, diabetes, burns, pains, during pregnancy, during estrogen therapy, etc. Cortisol blood level may be increased by intake of ACTH, Cortisol, alcohol, nicotine, oral contraceptives. Decreased Cortisol levels are found in Addison syndrome, adrenogenital syndrome, hypopituitarism. Some drugs may decrease Cortisol level in blood, such as: L-DOPA, dexamethasone, etc. Decreased Cortisol level during pregnancy may indicate anencephaly of the fetus.

PRINCIPLE

This test is based on competition enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to cortisol-antibodies simultaneously with conjugated Cortisol-peroxidase. Cortisol from the specimen competes with the conjugated Cortisol for coating antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell

surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is inversely related to the quantity of the measured analyte in the specimen.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C. Immediately after use remaining reagents should be returned to cold storage at 4˚C

MATERIALS SUPPLIED IN THIS KIT

1. Cortisol Strip Plates, 8 X 12 wells. Qty: 1
2. Calibrators: C1: 0, C2: 40, C3: 80, C4: 200, C5: 600, C6:2000 nmol/l, 6pcs
3. Control Serum, Qty: 1
4. Conjugate, Qty: 1
5. Substrate Solution, Qty: 1
6. Wash Buffer Concentrate 26X, Qty: 1
7. Stop Solution, Qty: 1
8. Plate Sealing Tape, Qty: 2
9. Instruction Manual, Qty: 1

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

• When mixing or reconstituting protein solutions, always avoid foaming.

• To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the

assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C. If samples are to be used within 24 hours, they may be stored at 2 to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

—All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.

—All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

—It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

—Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

ASSAY PROCEDURE

1 Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.

2 Pipet 25 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells.

3 Dispense 100 µl of conjugate solution into the wells. Cover the wells by plate adhesive tape (included into the kit).

4 Incubate 60 minutes at 37 °C.

5 Prepare washing solution by 26x dilution of washing solution concentrate (BUF

WASH 26X) by distilled water. Wash the strips 5 times.

6 Dispense 100 µl of SUBS TMB into the wells

7 Incubate 10–20 minutes at +18…+25 °C

8 Dispense 100 µl of STOP into the wells.

9 Measure OD (optical density) at 450 nm.

10 Set photometer blank on air

11 Apply lin-log method for data reduction.

EXPECTED VALUES AND CHARACTERISTICS

The following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, nmol/l	
	Lower limit	Upper limit
Healthy donors	140	600

PERFORMANCE CHARACTERISTICS

Sensitivity of the assay was assessed as being 12 nmol/l.

Linearity was checked by assaying dilution series of 5 samples with different cortisol concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known Cortisol concentrations. The recovery percentages ranged from 90 to 110%

Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
Cortisol	100
11-Deoxycortisol	0,9
Prednisolone	5,6
Corticosterone	0,6
11-Deoxycorticosterone	<0,1
Progesterone	<0,1
17-Hydroxyprogesterone	<0,1
Testosterone, Estradiol, Estriol	<0,1
Danazol	<0,01

CALCULATION

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples. Plot a calibration curve on graph paper: OD versus Cortisol concentration. Determine the corresponding concentration of Cortisol in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).