Instruction For Use

【Product Name】

Cortisol (eCLIA)

【Order Information】

REF NO.	Package Size
0320510301	50 T
0320510302	2×50 T
0320510303	100 T
0320510304	2×100 T

【Intended Use】

Immunoassay for the in vitro quantitative determination of cortisol in human serum and plasma. Determination of cortisol is used for auxiliary evaluation of adrenal cortex function.

【Summary】

Cortisol (hydrocortisone) is an adrenocortical hormone synthesized and secreted by the adrenal cortex. It regulates the metabolism of sugars, proteins, and fats, maintains normal blood pressure, and suppresses inflammation and allergies reactions. The hypothalamic secretion of corticotropin-releasing hormone (CRH) can cause the anterior pituitary gland to periodically secrete adrenocorticotropic hormone (ACTH). Increased ACTH production stimulates cortisol production. High concentrations of glucocorticoids in the blood inhibit CRH and ACTH secretion through a negative feedback mechanism that reduces cortisol levels[1-4]. Cortisol is mainly used in clinical evaluation of adrenal cortical function. The serum cortisol concentration has a circadian rhythm. The highest peak usually occurs in the early morning (700 nmol/L or 25.4 mg/dL) and then gradually decreases to about half the peak concentration by night[5]. It is therefore important to specify the time of blood collection when interpreting the results. Measurement of cortisol in patients can be used to evaluate whether the hypothalamic-pituitary-adrenal axis function is normal or not. Primary or secondary adrenal insufficiency can lead to the decrease of cortisol content. Addison's disease is caused by primary adrenal insufficiency due to metabolic disturbance or damage to the adrenal cortex. Destruction or loss of the pituitary gland function leads to loss of ACTH stimulation to the adrenal gland, resulting in secondary adrenal dysfunction[6]. Primary or secondary adrenal hyperfunction leads to a rise in cortisol levels that can trigger Cushing's syndrome. The etiology of primary adrenal hyperfunction is usually nodular adrenal hyperplasia and adrenal tumor. Secondary adrenal hyperfunction is usually caused by excessive ACTH production by the pituitary gland or ectopic ACTH production by the tumor. Cortisol levels can rise during pregnancy or stress caused by depression, trauma, low blood sugar, alcoholism, diabetes, and hunger[7]. Due to the cyclical nature of cortisol secretion throughout the day, the estimation of serum cortisol at a single time point is of no practical value. Cortisol is usually measured with a dynamic function test[6-7].

【Test Principle】

Competition principle. Total duration of assay: 9 minutes.

10 µL of sample is incubated with biotinylated cortisol antibodies. After addition of cortisol antigen labeled with ruthenium complex and streptavidin-coated microparticles, the cortisol antigen in the sample compete with rutheniumlated cortisol antigen for biotinylated cortisol antibodies. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with system reagent. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the RFID.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles, 0.45mg/mL; 0.1M PBS, ProClin300	2×1.8mL	1×1.8mL	1×3.5mL	2×3.5mL
(RB)	Biotinylated monoclonal cortisol antibodies, 360 ng/mL; 0.05 M PBS; ProClin300	2×4.0mL	1×4.0mL	1×8.0mL	2×8.0mL
(RA)	Cortisol antigen labeled with ruthenium, 100 ng/mL; 0.05 M PBS; ProClin300	2×4.0mL	1×4.0mL	1×8.0mL	2×8.0mL
Calibrators	Cortisol antigen; 0.05 M PBS; ProClin300	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Cortisol antigen; 0.05 M PBS; ProClin300	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability: This method can be traced to manufacturer's selected measurement procedure.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- [illegible]
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzers eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2ºC-8°C.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	8 weeks
on the analyzers at 2~15°C	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and Control Materials	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	8 weeks

Store calibrators and control materials upright in order to prevent the calibrator solution from adhering to the cap of the vial.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-K3, EDTA-K2, heparin lithium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 24 hours at room temperature (18~28ºC), 4 days at 2~8ºC, and 12 months at -15ºC or below. Freezing and thawing can be performed once.

Due to the biological rhythmicity of cortisol, the sampling time must be indicated.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Cortisol test according to the operational manual.

Put the Cortisol reagent pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Recommended ambient temperature:10~30°C; Relative humidity: ≤ 80%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 4 weeks;

(3) Quality control findings outside the defined limits.

(4) The Buffer of different lots are used.

Quality control

For quality control, use Cortisol Control Materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is nmol/L, μg/dL, μg/L.

Conversion factors: nmol/L × 0.03625 = μg/dL

nmol/L × 0.3625 = μg/L

Specimen dilution

Cortisol concentration in the sample higher than the upper limit of detection can be diluted manually with sample diluent.

The recommended dilution ratio is 1:10. The results are reported by multiplying the measured values by the dilution ratio.

【Biological reference interval】

By analyzing the serum samples of healthy population from hospitals, the calculation of reference interval is shown in the table below:

Blood sampling time	Number	Reference interval (nmol/L)
6:00~10:00	133	165.2 ~ 505.7
16:00~20:00	127	73.20 ~ 290.8

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 0.5 ~ 1750 nmol/L. If the Cortisol concentration in the sample is lower than the lower limit of detection, the result is reported as <0.5 nmol/L; if the Cortisol concentration in the sample is higher than the upper limit of detection, the result is reported as >1750 nmol/L, or the sample is manually diluted with sample diluent at the dilution ratio of 1:10, and the result is reported with the measured value multiplied by the dilution ratio.

When jaundice (bilirubin)≤ 25 mg/dL, hemolysis (hemoglobin) ≤ 500 mg/dL, lipemia (triglyceride) ≤ 1500 mg/dL, biotin ≤ 30 ng/mL, HAMA ≤ 100 ng/mL, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample ≤ 600 IU/mL, the relative recovery of the test results is between 90% and 110%.

Due to the circadian rhythm of cortisol secretion, the collection time of the specimen must be considered in the interpretation of the results. Too much stress can raise cortisol levels. Pregnancy, birth control drugs, and estrogen therapy can increase cortisol levels. Patients who used hydroprednisone, methylprednisolone, or prednisone had a spurious increase in test results. Metropine assay revealed increased levels of 11-dehydrocortisol. The spurious increase in cortisol may be due to cross-reaction. Patients with 21-hydroxylase deficiency also have elevated cortisol levels due to elevated levels of 21-deoxyhydrocortisone.

【Analytical Performance】

Lower limit of measurement

Limit of Blank = 0.5 nmol/L.

Limit of Detection = 1.5 nmol/L.

Accuracy

The relative deviation shall not exceed ±10%.

Linearity

The correlation coefficient is not less than 0.9900 in the interval of 1.5 ~ 1750 nmol/L.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than or equal to 8.0%.

Between-lot precision

The coefficient of variation (CV) is less than or equal to 10.0%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: The coefficient of variation (CV) is less than or equal to 8.0%.

Accuracy of calibrators

The relative deviation shall not exceed ±10%.

Measured values of control materials

The measured results of supporting assigned control materials of the kit should be within the quality control range.

Method comparison

A comparison of the Lifotronic Cortisol assay (y) with the Roche Elecsys Cortisol assay method (x) using clinical samples gave the following correlations:

Number of plasma samples measured: 107

Linear regression:

Y=0.988X+20.944

r = 0.987

The sample concentrations were between 2.25 and 1750 nmol/L.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

Exercise the normal precautions required for handling all laboratory reagents.

All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Literature References】

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3. Lin L, Achermann JC. The Adrenal. Horm Res 2004;62:22-29.
4. EngelmannM, Landgraf R, Wotjak CT. The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. Front Neuroendocrinol 2004;25:132-149.
5. Aron DC, Tyrell JB. Glucocorticoids & Adrenal Androgens. In: Greensoan FS, Baxter JD (eds), Basic & Clinical Endocrinology, 4th edition Appleton & Lange, USA 1994;307-346.
6. Chodosh LA, Daniels GH. Addison's disease. Endocrinologist 1993; 3(3):166-81.
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【Symbols Explanation】

Symbol	Title	Symbol	Title
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Contains sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limitation		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Manufacturer】

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【Version and Revision】

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