Instruction For Use

【Product Name】

ACTH (eCLIA)

【Order Information】

REF NO.	Package Size
0320510901	50 T
0320510902	2×50 T
0320510903	100 T
0320510904	2×100 T

【Intended Use】

Immunoassay for the in vitro quantitative determination of Adrenocortiotropic Hormone (ACTH) in human plasma. Determination of ACTH is used for evaluating pituitary-adrenal function.

【Summary】

Adrocorticotropin (ACTH) is a peptide hormone composed of 39 amino acids which synthesized in the anterior pituitary gland as part of the precursor molecule opioid-melanocytic cortitonogen (POMC). Tissue-specific cleavage produces ACTH and some related peptides. ACTH acts on the adrenal cortex to promote the synthesis and secretion of glucocorticoids (especially cortisol)[1-2]. Glucof corticoid oids is regulated by various factors. Upon stimuli such as physiological effects or the body clock, the hypothalamus secrete CRH (corticotropin-releasing hormone). CRH acts on the pituitary gland for the synthesis and secretion of ACTH. Finally, ACTH stimulates the secretion of glucocorticoids from the adrenal glands. High concentrations of glucocorticoids in the blood can inhibit the secretion of both CRH and ACTH through a negative-feedback mechanism[3-6]. ACTH concentrations showed a diurnal variation, being high in the morning and low at night. Therefore, it is important to understanding the timing of plasma samples for explain the detection results of ACTH. The results of ACTH in plasma play an important role in identifying Cushing's disease (ACTH hypersecretion), pituitary tumors that spontaneously produce ACTH (e.g. Nelson syndrome), hypopituitarism associated with ACTH deficiency, and ectopic ACTH syndrome.

【Principles of the testing method】

Sandwich principle. Total duration of assay: 9 minutes.

60 µL of sample, biotinylated monoclonal antibody, and monoclonal antibody labeled with ruthenium complex form a sandwich complex. After addition of streptavidin-coated microparticles, the 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 magnetic particles, 0.45 mg/mL, 0.1M PBS, ProClin300	2×1.8mL	1×1.8mL	1×3.5mL	2×3.5mL
(RB)	Biotinylated monoclonal ACTH antibodies,0.1M HEPES; ProClin300	2×3.5mL	1×3.5mL	1×7.0mL	2×7.0mL
(RA)	Monoclonal ACTH antibodies labeled with ruthenium,0.1M HEPES; ProClin300	2×3.5mL	1×3.5mL	1×7.0mL	2×7.0mL
Calibrators	ACTH antigen, lyophilized; ProClin300	High: 1×4.0 mL Low: 1×4.0 mL			
Control Materials (Optional)	ACTH antigen, lyophilized; ProClin300	High: 2×4.0 mL Low: 2×4.0 mL			

The calibration process of this kit is traceable to the manufacturer's selected measurement procedures.

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

【Instruments and Materials Required but Not Provided】

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

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- 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 eCL8000, 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 Analyzer 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

【Applicable Instrument】

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

【Storage and Shelf Life】

Store at 2-8℃.

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℃	18 months
After opening Store at 2~8℃	8 weeks
Stored on the analyzers at 2℃~15℃	8 weeks

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

Stability of the calibrators and control materials	
Lyophilize unopened at 2~8℃	18 months
Reconstituted store at 2~8℃	2 hours
Reconstituted on the analyzer at 18 ℃-28 ℃	Use it immediately and only once
Reconstituted stored at -15℃ or lower than -15℃	2 months (freeze-thawed only once)

*The calibrators and control materials are lyophilized, which should be separated and frozen as soon as possible after remelting.

【Specimen collection, handling and storage】

Human EDTA-K3、EDTA-K2 plasma are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended.

Samples can be stored for 8 hours at 18℃-28 °C, 4 weeks at -15℃ or lower than -15℃. The samples may be freeze-thawed once.

Ensure the samples and calibrators are at 18℃-28 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

【Testing 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 ACTH test according to the operational manual.

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

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

Pretreatment of lyophilized calibrators and control materials:

Carefully dissolve the contents of calibrator by adding exactly 4.0 mL of distilled or deionized water and allow to stand closed for 10 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators or control materials to the additional bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15℃ or lower than -15℃.

Carefully dissolve the contents of control material by adding exactly 4.0 mL of distilled or deionized water and allow to stand closed for 10 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators or control materials to the additional bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15℃ or lower than -15℃.

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 lot are used.

Quality control procedure

For quality control, use ACTH 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.

Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is pg/mL, pmol/L or ng/L.

Conversion factors: pg/mL x 0.2202 = pmol/mL

pg/mL = ng/L

【Biological reference interval】

By analyzing the plasma samples of healthy population, the calculation of percentile point of 5th and 95th values as reference interval is 7.172~64.03 pg/mL.

Plasma samples will need to be collected at 7~10 am.

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 measuring range of the kit is 0.2~2000 pg/mL. For samples with ACTH content below the lower detection limit, the results are reported as < 0.2 pg/mL; If the ACTH content is above the upper detection limit, the results are reported as > 2000 pg/mL.

When jaundice (bilirubin) is ≤25 mg/dL, hemolysis (hemoglobin) is ≤0.4 g/dL, lipemia (triglyceride) is ≤1500 mg/dL, biotin is ≤60 ng/mL and HAMA ≤100 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is less than or equal to 400 IU/mL, the relative recovery of the test results is between 90% and 110%.

When the ACTH concentration reaches 1×10^{6} pg/mL, there is no high-dose Hook effect.

【Analytical Performance】

Lower limit of measurement

Limit of Blank= 0.2 pg/mL

Limit of Detection= 1pg/mL.

Accuracy

The relative deviation shall not exceed ±10%.

Linearity

The correlation coefficient is not less than 0.9900 in the interval of 1~2000 pg/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than or equal to 8%.

Between-lot precision

The coefficient of variation (CV) is less than or equal to 10%.

Homogeneity of calibrators and control materials

Between-bottle homogeneity: The coefficient of variation (CV) is less than or equal to 10%.

Accuracy of calibrators

The relative deviation shall not exceed ±10%.

Measured values of quality control

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

Method comparison

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

Linear regression:

Y=0.9746X+2.6938

r = 0.996

The sample concentrations were between 5.11 and 1905 pg/mL.

【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.

【References】

1. Cone RD. Anatomy and regulation of the central melanocortin system. Nature Neurosci 2005;8:571-578.
2. Talbot JA, Kane JW, White A. Analytical and clinical aspects of adrenocorticotrophin determination. Ann Clin Biochem 2003;40:453-471.
3. Jacobson L. Hypothalamic-pituitary-adrenocortical axis regulation.Endocrinol Metab Clin North Am 2005;34:271-292.
4. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism,and action. Endocrinol Metab Clin North Am 2005;34:293-313.
5. Lin L, Achermann JC. The Adrenal. Horm Res 2004;62:22-29.
6. 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.

【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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