Instruction For Use

【Product Name】

C-peptide (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
695022	50T
695023	2×50T
695011	100T
695021	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of C-peptide in human serum and plasma.

C-peptide is a single-chain polypeptide consisting of 31 amino acids with a molecular weight of about 3021 Daltons[1,2]. In islet beta cells, a pre-proinsulin of 105 amino acid residues is first synthesized, then proteolytically divided into a long peptide chain of 86 amino acids - proinsulin. Proinsulin enters the Golgi apparatus with microbubbles in the cytoplasm. By action of enzymatically cleaving Arg^{31}-Arg^{32} and Arg^{60}, the forming C-peptide and insulin, are secreted into B cells, then, enter into blood circulation. As the concentration of proinsulin is less than 10 % of C-peptide, free C-peptide thus represents total C-peptide (i.e. C-peptide fragment in proinsulin plus C-peptide) in the blood. Insulin breaks down in the liver and kidney while the intact C-peptide is excreted from the kidney.

Recent studies show C-peptide is a biologically active polypeptide. Evidence indicates that C-peptide as a substitute for insulin contributes to prevent progression of long-term complications of type 1 diabetes[3,4].

The determination of C-peptide can be used to understand the function of islet cells, which is of great significance for the diagnosis and treatment of diabetes:

(1) C-peptide reflects the secretory function of the islet ß cells in the body, especially in patients treated with exogenous insulin. For diabetic patients treated with insulin, sometimes accompanied by islet cell tumor or ß-cell hyperplasia, leading to severe hypoglycemia. Determination of C-peptide helps to identify whether it is caused by ß-cell lesions.

(2) C-peptide determination has guiding significance for the classification of diabetic patients and the identification of hypoglycemia. Determination of C-peptide levels in urine and fasting contributes to the classification of diabetic patients and distinguishes insulin-dependent patients from non-insulin-dependent patients.

(3) Determination of C-peptide can be used as a quantitative indicator to identify the efficacy of pancreatic surgery and the residual ß-cell secretion function to determine whether insulin is administered. It's also beneficial to determine in the follow-up whether the tumor has recurrence or metastasis.

(4) C-peptide determination is more applicable in hepatopathy patients. In hepatitis or cirrhosis, the liver decreases insulin intake, thus, blood insulin levels increase, while C-peptide is less affected, and the ratio of C-peptide to insulin in blood is decreased.

【Test Principle】

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: 25 µL of sample, biotinylated monoclonal C-peptide antibody and monoclonal C-peptide antibody labeled by ruthenium trisbipyridyl NHS ester form a sandwich compound by immunoreaction.

2nd incubation: After addition of streptavidin-coated micro magnetic beads, the compounds bind to the micro beads via interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the micro beads and immuno-compounds are magnetically captured onto the surface of the electrode.

Unbound substances are then removed by Buffer. Application of a voltage to the electrode then induces emission of luminescence which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

【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 (1×50T)	Volume (2×50T)	Volume (1×100T)	Volume (2×100T)
(MB)	Streptavidin-coated micro magnetic beads, 0.75 mg/mL: 0.05 % ProClin™ 300	1×3.0 mL	2×3.0 mL	1×5.0 mL	2×5.0 mL
(RB)	Anti-C-peptide-Ab~biotin, 1.5 mg/L: 50 mM MES; 0.05 % ProClin™ 300	1×5.5 mL	2×5.5 mL	1×9.0 mL	2×9.0 mL
(RA)	Anti-C-peptide-Ab~$Ru(bpy)_3^{2+}$, 1.5 mg/L: 50 mM MES; 0.05 % ProClin™ 300	1×5.5 mL	2×5.5 mL	1×9.0 mL	2×9.0 mL
Calibrators	Bovine serum product,0.05 % ProClin™ 300; lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Bovine serum product,0.05 % ProClin™ 300; lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			

This method can be traced back to the WHO International Reference Material for C-peptide (13/146).

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

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

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 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 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

【Storage and Shelf Life】

Store at 2ºC-8ºC.

Do not freeze.

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

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
Stored on the analyzers at 2ºC~15ºC	8 weeks

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

Stability of the calibrators and control materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after reconstituted	7 days
Store at -25ºC~-15ºC after reconstituted	90 days (freeze-thawed only once)

【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 serum samples or plasma samples collected from EDTA and heparin anticoagulant blood collection tubes.

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 would better to be tested in 4 hours after collection, otherwise, should be stored 2~8ºC for no more than 24 hours or -25~-15ºC, 90 days. Freezing and thawing cycle is permitted only once.

【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 C-peptide test according to the operational manual.

Put the C-peptide 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%.

Handing of lyophilized calibrators and control materials:

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 10~15 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°C or lower than -15°C.

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 (RFID) 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:

(2) The same lot of reagents were used on the analyzer beyond 28 days;
(3) Quality control misses the target.

Quality control

For quality control, use C-peptide control materials.
In addition, other suitable control material can be used.
In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.
Pre-treatment of lyophilized calibrator and quality control:
Accurately add 1.0 mL of deionized water, and cover it at room temperature for 10 to 20 minutes to reconstitute the contents of the bottle. Mix well and avoid air bubbles. The calibrated product or control product after mixing is marked and dispensed. If you do not test immediately, you need to quickly transfer to -25~-15ºC storage.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL or nmol/L : 1 ng/mL = 0.33333 nmol/L, or 1 nmol/L = 3 ng/mL.

Specimen dilution

Samples with C-peptide concentrations above the measuring range would be manually diluted with C-peptide-negative serum.
The recommended dilution ratio is 1:10, the concentration of a diluted sample must be >4 ng/mL. After manual dilution, multiply the result by the dilution factor.

【Biological reference interval】

According to serum test results of 419 healthy human serum samples (males 203, females 216) in Guangdong Province, P. R. China, the percentile 5 to 95 gives the reference range of 1.06~4.81 ng/mL.
Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
The measuring range of the kit is 0.01~40 ng/mL. For samples with C-peptide content below the lower detection limit, the results are reported in < 0.01 ng/mL; If the C-peptide content is above the upper detection limit, then the sample should be diluted and re-tested. The results are reported after multiplying with the dilution factor.
There is no high-dose hook effect at C-peptide concentration 200 ng/mL.
When the sample containing bilirubin ≤ 60 mg/dL, triglyceride ≤ 2000 mg/dL, hemoglobin ≤ 200 mg/dL, biotin ≤ 30 ng/mL, HAMA ≤ 120 ng/mL, interference deviation to the test result lies within ±10 %. The test result would not be interfered by rheumatoid factor (1500 IU/mL).

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 0.01 ng/mL.

Accuracy

When measuring the international reference material (NIBSC 13/146, WHO) or metrologically traceable accuracy control materials, the ratio between resulting value and target value lies between 0.900 and 1.100.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 0.01~40 ng/mL.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 8 %.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10 %.

Analytical specificity

Detection of C-peptide analogs,the test results are as follows:

Analogs	Concentration of Analogs	Cross Reactivity
ProInsulin	100 ng/mL	≤30.0 %
Human insulin	8.66 μg/mL	≤0.001 %
Porcine insulin	7.50 μg/mL	≤0.001 %
Bovine insulin	7.69 μg/mL	≤0.001 %
Insulin-like growth factor I	1 μg/mL	≤0.001 %
Human growth hormone	10 μg/mL	≤0.001 %
Glucagon	10 μg/mL	≤0.001 %

Homogeneity of calibrators and control materials

In-bottle homogeneity: coefficient of variation (CV) ≤ 10 %.
Between-bottle homogeneity: coefficient of variation (CV) ≤ 10 %.

Method comparison

A comparison of the Lifotronic C-peptide assay (y) with the Roche Elecsys C-peptide Method (x) using clinical samples gave the following correlations:
Linear regression:
y=0.9979x+0.0133
r = 0.9976
The sample concentrations were between approx 0.297 and 35.72 ng/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.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Batch code		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number		Material code
	Warning		Date of manufacture
	Unique device identifier		

【Reference】

1. Clark PM. Assays for insulin, proinsulin(s) and C-peptide. Ann Clin Biochem 1999;36(5):541-564.
2. Sacks DB. Chapter 24: Carbohydrates. In: Burtis CA, Ashwood ER (eds). Tietz Textbook of Clinical Chemistry, WB Saunders,Philadelphia, 3rd edition; 1999:750-808.
3. Johansson J, Ekberg K, Shafqat J, Henriksson M, Chibalin A,Wahren J, Jörnvall H. Molecular effects of proinsulin C-peptide. Biochem Biophys Res Commun 2002;295:1035-1040.
4. Forst T, Rave K, Pfuetzner A, Buchholz R, Pohlmann T, Löbig M,Heinemann L. Effect of C-Peptide on Glucose Metabolism in Patients With Type 1 Diabetes. Diabetes Care 2002;25 (6):1096-1097.

【Manufacturer】

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【Version and Revision】

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