Instruction For Use

【Product Name】

Anti-HCV (eCLIA)

【Order Information】

REF NO.	Package Size
0320508907	50T
0320508912	2×50T
0320508908	100T
0320508913	2×100T

【Intended Use】

Immunoassay for the in vitro qualitative determination of antibodies to hepatitis C virus (HCV) in human serum and plasma. The determination of Anti-HCV is utilized for auxiliary diagnosis of hepatitis C virus infection, for screening of blood donations and screening test to prevent transmission of hepatitis C virus.

【Summary】

The hepatitis C virus (HCV) is a small enveloped RNA virus belonging to the family flaviviridae and genus hepacivirus. The HCV RNA genome is 9,600 nucleotides in length and encodes a single polyprotein that is post-translationally cleaved into10 polypeptides including encoding 3 structural (Core, Envelope 1 and 2) and 7 non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins[1]. The virus was first identified in 1989[2], and HCV infection is a blood-borne disease that infects ~185 million people (~3% of the world's population) worldwide[3]. The highest prevalence is found in Africa, the Mediterranean and Asia. It can result in severe liver disease and is the leading cause of liver cancer in many countries. Approximately 350,000 deaths each year occur as a result of HCV infections. Chronic hepatitis C is leading causes of cirrhosis and hepatocellular carcinoma (HCC), which ranks as the third cause of cancer deaths worldwide[4]. HCV chronic infection can lead to chronic inflammatory necrosis or fibrosis of the liver, and some patients can develop cirrhosis or even hepatocellular carcinoma[5]. It does great harm to the health and life of patients and has become a serious social and public health problem. There is no effective vaccine for hepatitis C[6]. Transmission of HCV occurs by percutaneous exposure to blood, blood products, or organs from an infected person. In developed regions where blood donor screening programs have operated for many years the major mode of HCV transmission is through intravenous drug use. In less developed regions, the major routes of transmission are through medical treatment with unsterilized equipment or unscreened blood. Therefore, control hepatitis C can only from the source of infection and transmission way, early accurate diagnosis and discovery of HCV infection is the most effective means of blocking hepatitis C infection. Anti-HCV is the antibody that human immune cell makes response to infection of hepatitis C virus. Immunological methods can be used to qualitatively detect Anti-HCV in human serum or plasma for auxiliary diagnosis of hepatitis C virus infection.

【Test Principle】

Sandwich principle. Total duration of assay:18 minutes.

50 μL of sample, biotinylated HCV-specific antigens and HCV-specific antigens labeled with ruthenium complex react to 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 automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

【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 (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles 0.45mg/mL; 0.1M PBS, ProClin300.	1×1.75 mL	2×1.75 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Biotinylated HCV-specific antigens, 0.5mg/L; 20 mM PBS, ProClin300.	1×2.75 mL	2×2.75 mL	1×5.5 mL	2×5.5 mL
Reagent A (RA)	HCV-specific antigens labeled with ruthenium 0.5mg/L; 20 mM PBS, ProClin300.	1×2.75 mL	2×2.75 mL	1×5.5 mL	2×5.5 mL
Anti-HCV Calibrators	Negative calibrator, non reactive for anti-HCV antibodies, horse serum, ProClin300.	Cal 1: 1×1.5 mL			
	Positive calibrator, reactive for anti-HCV antibodies, horse serum, ProClin300.	Cal 2: 1×1.5 mL			
Anti-HCV Controls (Optional)	Negative control, non reactive for anti-HCV antibodies; horse serum; ProClin300.	Level 1: 1×1.5 mL			
	Positive control, reactive for anti-HCV antibodies; horse serum; ProClin300.	Level 2: 1×1.5 mL			

Accuracy: This method can be traced to manufacturer's selected measurement procedures..

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 Series 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 Analyzers Series 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 Series 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, o 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.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened 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 are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
Stored on the analyzers at 18ºC~28ºC	8 hours

Store calibrators and control materials upright in order to prevent the calibrators and control materials 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】

Human serum and plasma are recommended. Blood samples should be collected by standard operation of venous puncture. Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel. Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, sodium citrate plasma tubes containing separating gel. 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 can be stored for 7 days at 18 ºC-28 ºC, 14 days at 2 ºC-8 ºC, 3 months at -20 ºC ± 5 ºC. Freezing and thawing cycle is permitted 6 times.

Ensure the samples, calibrators and control materials are at 18 ºC-28 ºC prior to measurement.

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

【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 Anti-HCV test according to the operational manual.

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

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

Calibration

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

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended when:

(1) The reagents of different lots are used;

(2) The same reagent lot was used on the analyzer beyond 28 days;

(3) Quality control is beyond the defined limits;

(4) The Buffer of different lots are used.

Quality control
Use Anti-HCV Controls for quality control.
In addition, other suitable Control Materials are also applicable.
Controls 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 analyzer automatically calculates the cutoff based on the measurement of Anti-HCV Cal 1 and Anti-HCV Cal 2.
The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI).

【Result Interpretation】

Initial Results		
Concentration	Instrument Result	Retest Procedure
< 1.0 COI	Non-reactive	Not retest required
≥ 1.0 COI	Reactive	Retest in duplicate

Duplicate Retest Results	
Instrument Result	Specimen Result
both results non-reactive	Specimens are considered non-reactive for Anti-HCV
one or both reactive	Specimen are considered repeatedly reactive for Anti-HCV

Repeatedly reactive samples are recommended for further investigating by supplemental methods (e.g. immunoblot or detection of HCV RNA).

【Limitations】

Interference
The effect of the following endogenous substances and pharmaceutical compounds on assay perfomance was tested.
Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances
When the sample containing bilirubin ≤ 66 mg/dL, triglyceride ≤ 2000 mg/dL, Human serum albumin ≤ 10 g/dL, biotin ≤ 42 ng/mL, or hemoglobin ≤ 1.0 g/dL, or rheumatoid factors ≤ 1200 IU/mL and HAMA ≤ 250 ng/mL, the test result would not be interfered.
No false negative result due to high-dose hook effect was found with the Anti-HCV (eCLIA).

Pharmaceutical substances
In vitro tests were performed on commonly used pharmaceuticals and drugs (5.34 μg/mL zidovudine, 4.59 μg/mL lamivudine, 5.74 μg/mL tenofovir, 6.31 μg/mL sustiva, 12.58 μg/mL lopinavir, 5.73 μg/mL abacavir, 12.71 μg/mL tenofovir disoproxil fumarate, 4.95 μg/mL emtricitabine, 5.33 μg/mL nevirapine, 7.33 μg/mL edurant, 14.42 μg/mL ritonavir, 10.95 μg/mL Prezista, 8.89 μg/mL isentress, 14.24 μg/mL indinavir sulfate, 0.18 mg/L Peginterferon alfa-2a, 20 IU/L Interferon alfa, 1200 mg/L ribavirin) used in HCV therapy. No interference with the assay was found.

【Analytical Performance】

Specific performance data
Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision
The precision of Anti-HCV are the Repeatability(%CV) ≤8%, the Within-Laboratory Precision(%CV) ≤10%, the Reproducibility(%CV) ≤10%.
Precision was determined using reagents, samples in a protocol (EP5-A3) of the CLSI (Clinical and Laboratory Standards Institute). The following results:

Sample	Mean (COI)	Repeatability (CV)	Within-Laboratory Precision (CV)	Reproducibility (CV)
Human Sample, negative	0.582	1.74%	5.11%	3.78%
Human Sample, weakly positive	1.815	1,.49%	4.27%	2.87%
Human Sample, positive	5.158	1.29%	4.44%	2.39%

Homogeneity of calibrators and control materials
Within-bottle homogeneity: the coefficient of variation (CV) ≤ 8.0%.
Between-bottle homogeneity: the coefficient of variation (CV) ≤ 10.0%.

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

Analytical specificity
Samples containing potentially interfering substances or derived form high-risk groups were tested with the assay. The specimens containing antibodies against HAV, HBV, HIV, HEV, HSV, HBsAg, CMV and Toxoplasma gondii, Rubella virus, Treponema pallidum, IgG, IgM, IgA, Escherichia coli and EB virus, elevated titers of rheumatoid factor, elevated titers of HAMA were tested, no cross reaction was observed.

Diagnostic sensitivity
Samples from HCV infected patients with different stages of disease and infected with different HCV genotypes, Unselected blood donors, Hospitalised patients. The sensitivity was 100%. The 95% confidence lower limit was 99.27%.

Diagnostic specificity
Samples from HCV infected patients with different stages of disease and infected with different HCV genotypes, Unselected blood donors, Hospitalised patients. The specificity of the assay was 99.96%. The 95% confidence lower limit was 99.86%.

【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 and calibrators should avoid foaming before and during test.
Safety data sheet available for professional user on request.
This product contains animal-sourced materials and may have potential biological risk. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with 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】
1. Hoofnagle JH. Course and outcome of hepatitis C. Hepatology 2002;36:21-29.
2. Choo QL , Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived form a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359-362.
3. Andrea L. Cox. Global control of hepatitis C virus. Science 2015;349, 790 .
4. Hatzakis A, Wait S. The state of hepatitis B and C in Europe: report form the hepatitis B and C summit conference. Journal of Viral Hepatitis 2011; 18(Suppl.1),1-16.
5. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997;26(Suppl 1):34-38.
6. Abdel-Hamid M, Ei Daly M, E1 Kafrawy S, Mikhail N, Strickland GT, Fix AD. Comparison of second-and third-generation enyme immunoassays for detecting antibodies to hepatitis C virus. J Clin Microbiol 2002;40(5):1656-9.

【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 limit		This way up
	Catalogue number	P/N	Material code
	Warning		Date of manufacture
	Unique device identifier		

【Manufacturer】
Shenzhen Lifotronic Technology Co., Ltd.
Lifotronic Tower, No. 8, Qiuzhi East Road, Guancheng Community, Guanhu Street, Longhua, 518110 Shenzhen, PEOPLE'S REPUBLIC OF CHINA
E-mail: inter-service@lifotronic.com

【Version and Revision】
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