Instruction for Use

【Product Name】

Alpha-Fetoprotein (eCLIA)

【Order Information】

REF NO.	Package Size
0320501401	50T
0320501402	2×50T
0320501403	100T
0320501404	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of alpha-fetoprotein (AFP) in human serum or plasma samples, and clinically mainly used for the assisted diagnosis, efficacy determination and prognosis observation of primary liver cancer.

【Summary】

Alpha-Fetoprotein (AFP) is a glycoprotein composed of 591 amino acids synthesized by liver and yolk sac in early fetal development, with the molecular weight of about 70000 Daltons, which is located between albumin and α1 globulin in electrophoresis[1, 2]. AFP is a clinical marker of hepatocellular carcinoma (HCC) and germ cell tumors, which is positive in about 70-95% of primary HCC[3]. AFP is very high in the neonatal period, and drops to 10 μg/L ~ 20 μg/L at the age of one year. The content of AFP in adult serum is very low. When the hepatocytes become malignant, the AFP content increases obviously, which is an important indicator for clinical diagnosis of primary liver cancer[4].

The change of AFP concentration in human serum is of great value for efficacy observation and cancer recurrence of patients with liver cancer. It is reported that over 70%-80% of patients with primary liver cancer have elevated serum AFP level[5, 6].

It can be used as an indicator to test liver cancer, but the level of AFP has no absolute relationship with the size of liver cancer, so it cannot be used as the basis for early diagnosis or confirmed diagnosis of malignancies, and it is not recommended to be used for screening and early diagnosis of malignancies.

【Test Principle】

Sandwich principle. Total duration of assay: 9 minutes.

10 μL of sample, biotinylated monoclonal AFP antibodies, and monoclonal AFP antibodies labeled with ruthenium complex react to form a sandwich complex. 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; ProClin™300	2×1.3 mL	1×1.3 mL	1×2.5 mL	2×2.5 mL
(RB)	Biotinylated monoclonal AFP antibodies, 0.2 mg/L; 0.05M MES, ProClin™300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
(RA)	Monoclonal AFP antibodies labeled with ruthenium; 0.4 mg/L; 0.05M MES, ProClin™300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
Calibrators	AFP antigen, 0.1 M PBS, ProClin™300	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	AFP antigen, 0.1 M PBS, ProClin™300	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability:This method has been standardized against the international standard substances NIBSC 72/225.

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
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated
- 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-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 store at 2~8°C	18 months
Store at 2~8°C after opening	8 weeks
Stored 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 Store 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 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 serum samples or plasma samples collected from EDTA-3K, EDTA-2K, heparin lithium and heparin sodium 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 4 days at 18~28ºC, 7 days at 2~8ºC, and 6 months at -15ºC or below. Freezing and thawing can be performed three times.

【Assay Procedure】

Testing procedure 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 AFP test according to the operational manual.

Put the AFP 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 28 days;

(3) Quality control findings outside the defined limits.

(4) The Buffer of different lots are used.

Quality control

For quality control, use AFP 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.

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 IU/mL or ng/mL, and the conversion relationship is IU/mL × 1.21 = ng/mL.

Specimen dilution

AFP concentration in the sample higher than the upper limit of detection can be diluted with sample diluent.
The recommended dilution ratio is 1:50 (automatic dilution by instrument or manual dilution). If manual dilution, the result should be multiplied by the dilution ratio. If instrument automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Interval】

By analyzing the serum samples of 256 healthy females and 248 healthy males from hospitals , it was calculated that the upper limit of the reference range of 95% is 5.75 IU/mL.
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 IU/mL~1000 IU/mL. If the AFP concentration in the sample is lower than the lower limit of detection, the result is reported as < 0.5 IU/mL; if the AFP concentration in the sample is higher than the upper limit of detection, the result is reported as > 1000 IU/mL (50× diluted sample is reported as > 50000 IU/mL).
When jaundice (bilirubin) is ≤ 20 mg/dL, hemolysis (hemoglobin) is ≤ 500 mg/dL, lipemia (triglyceride) is ≤ 520 mg/dL, biotin is ≤ 20 ng/mL, HAMA is ≤ 100 ng/mL in the sample, the interference deviation of the measurement results is within ±10%.
When the concentration of rheumatoid factor in the sample is ≤ 1500 IU/mL, the relative recovery of the test results is between 90% and 110%.
When the sample contains cyclophosphamide (25 mg/dL), acetaminophen (200 μg/mL), cisplatin (100 μg/mL), acetylsalicylic acid (0.5 mg/mL) and ascorbic acid (1000 μg/mL), respectively, the interference deviation of the measured results is within ±10%.
Samples of other tumor markers containing 1000 U/mL cancer antigen 125, 1000 ng/mL carcinoembryonic antigen, 100 U/mL cancer antigen CA15-3, 1000 U/mL carbohydrate antigen CA19-9, 100 ng/mL prostate-specific antigen and 1000 ng/mL ferritin are tested, and the AFP measurement results are not greater than 0.5 IU/mL.
When the concentration of AFP reaches 500000 IU/mL, the test results are not affected by hook effect.

【Analytical Performance】

Lower limits of measurement

Limit of Blank = 0.2 IU/mL.
Limit of Detection = 0.5 IU/mL.
Limit of Quantitation = 0.5 IU/mL.

Accuracy

The national standard is tested, and the relative deviation of the test results of is within ±10%.

Linearity

Within the range of 0.5 IU/mL ~ 1000 IU/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 8%.

Between-lot precision

The coefficient of variation (CV) ≤ 10%.

Accuracy of calibrators

The supporting calibrators of the kit are tested, the relative deviation of the test results of is within ±15%.

Measured values of control material

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

Homogeneity of calibrators and control materials

In-bottle homogeneity: coefficient of variation (CV) ≤ 8%.
Between-bottle homogeneity: coefficient of variation (CV) ≤ 10%.

Method comparison

A comparison of the Lifotronic AFP assay (y) with the Roche Elecsys AFP Method (x) using clinical samples gave the following correlations:
Number of serum samples measured:108
Passing-Bablok regression:
y=-0.236+ 0.989x
The sample concentrations were between approx 1.34 and 929.5 IU/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	IVD	In vitro diagnostic medical device
LOT	Batch code		Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		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		

【Reference】

1. Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest 1956;8:174.
2. Ruoslathi E, Engvall E, Kessler MJ. Chemical Properties of Alpha-Fetoprotein. In: Herberman RB, McIntire KR (eds). Immunodiagnosis of Cancer. New York: Marcel Dekker Inc 1979:101-117.
3. Ramsey WH, Wu GY. Hepatocellular carcinoma: update on diagnosis and treatment. Dig-Dis 1995;13,2:81-91.
4. Sato Y, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. New Engl J Med 1993;328,25:1802-1806.
5. Abelev GI. Alpha-fetoprotein as a model for studying reexpression of embryonic antigens in neoplasia. In: Herberman RB, McIntire KR, eds. Immunodiagnosis of Cancer. New York: Marcel Dekker, Inc., 1979: 76-101.
6. Stuart KE, Anand AJ, Jenkins RL. Hepatocellular Carcinoma in the United States. Prognostic features, treatment outcome, and survival. Cancer 1996; 77,11:2217-2222.

【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

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel. +49-40-2513175 Fax. +49-40-255726

【Version and Revision】

Version: 2.0
Issue Date: 2025-03-04