Instruction for Use

【Product Name】

Hyaluronic Acid (eCLIA)

【Order Information】

REF NO.	Package Size
692027	50T
692028	2×50T
692029	100T
692030	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of Hyaluronic Acid in human serum. The results can be used in assisting diagnosis of liver fibrosis.

Hyaluronic Acid (HA) is a component of proteoglycan in the extracellular matrix of liver, which is mainly synthesized in intrahepatic mesenchymal cells. It enters into blood circulation through lymphocytes, is quickly absorbed and metabolized into acetic acid and lactic acid by hepatic endothelial cells.

HA is a linear polysaccharide composed of D-glucuronic acid group and N-acetylglucosamine disaccharide unit repetition with molecular weight of 10-104kD[1].

HA is mainly metabolized in the liver. In patients with liver disease, especially with liver cirrhosis, the concentration of HA in blood is significantly increased due to the increased level of HA synthesis in liver fibroblasts and the obstruction of HA clearance in liver endothelial cells[2]. Serum HA is the most sensitive and reliable biochemical index to reflect the degree of liver disease and fibrosis. At the same time, it can roughly reflect the clinical effect of liver fibrosis treatment[3].

【Principles of the examination method】

Competition method. Total duration of assay: 18 minutes.

The determination of Hyaluronic Acid adopting competition method.

60 µL of samples and biotin-labeled HA derivatives compete to bind free Hyaluronic Acid Binding Proteins (HABP) to form biotin-labeled HA-HABP binding compounds. The binding compound and ruthenium-labeled HABP antibody produce an immune response, forming the biotin-labeled HA-HABP-ruthenium-labeled HABP antibody complex, which binds to streptavidin-coated magnetic beads and is aspirated into the measuring cell where the magnetic beads are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission, and the luminescence intensity is inversely proportional to HA concentration.

【Main Components】

The reagent pack consists of MB, RA, RB, RC, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	streptavidin-coated magnetic beads, 0.75 mg/mL; ProClin™ 30	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
(RB)	Biotin-labeled HA derivatives 0.5mg/L; 0.02M BIS-TRIS buffer; ProClin™ 30	2×2.5 mL	1×2.5 mL	1×5.0 mL	2×5.0 mL
(RA)	Ruthenium-labeled HABP monoclonal antibody 0.5mg/L; 0.02M BIS-TRIS buffer;ProClin™ 30	2×2.5 mL	1×2.5 mL	1×5.0 mL	2×5.0 mL
(RC)	Free bovine HABP 2mg/L; 0.02M BIS-TRIS buffer; ProClin™ 30	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
Calibrators	HA synthetic antigen, 0.1 M PBS, ProClin™ 30	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	HA synthetic antigen, 0.1 M PBS, ProClin™ 300	High: 1×2.0 mL Low: 1×2.0 mL			

Traceability: This method can be traced back 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 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 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 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
Store at 2℃~8℃ after opening	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	
Unopened store at 2℃~8℃	18 months
Store at 2℃~8℃ after opening	8 weeks

【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. Blood samples should be collected following standard venipuncture procedures. 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 (18~28℃), 48 hours at 2~8℃, and 30 days at -15℃ or below. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after one freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. HA testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30℃; relative humidity: ≤ 80%.

Calibration

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

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 HA 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 ng/mL.

【Biological Reference Interval】

By analyzing the serum samples of healthy people from hospitals, it was calculated that the upper limit of the reference range of 95% is 106.5 ng/mL.

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

【Result Interpretation】

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 5 ng/mL ~ 2000 ng/mL. If the HA concentration in the sample is lower than the lower limit of detection, the result is reported as < 5 ng/mL; if the HA concentration in the sample is higher than the upper limit of detection, the result is reported as > 2000 ng/mL.

When jaundice (bilirubin) ≤ 10 mg/dL, hemolysis (hemoglobin) ≤ 200 mg/dL, lipemia (triglyceride) ≤ 1000 mg/dL, biotin ≤ 10 ng/mL, total protein ≤ 4 g/dL, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is ≤ 800 IU/mL, the relative recovery of the test results is between 90% and 110%.

Samples containing 100 ng/mL LN, 100 ng/mL PIIINP, 1000 ng/mL CIV are tested, and the HA measured results are not greater than 10 ng/mL.

【Analytical Performance】

Lower limits of measurement

Limit of detection (LoD) ≤ 5 ng/mL.

Accuracy

The relative bias of measuring trueness control materials within ± 15%.

Linearity

Within the range of 5 ng/mL ~ 2000 ng/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run precision (repeatability)

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

Between-lot precision

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

Accuracy of calibrators

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

Homogeneity of calibrators and control materials

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

Method comparison

A comparison of the Lifotronic HA assay (y) with the Mindray HA reagent kit (x) using clinical samples gave the following correlations:
Number of serum samples measured: 116
Linear regression:
y=0.9578x+1.1600
r = 0.9917
The sample concentrations were between 5.52 and 1925.98 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	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	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		

【Literature References】

1. J.W. Kuo. Practical Aspects of Hyaluronan Based Medical Products CRC/Taylor & Francis, Boca Raton. 2006.
2. Pares A, Deulofeu R, Gimenez A, Caballeria L, Bruguera M, Caballeria J, Ballesta AM, Rodes J: Serum hyaluronate reflects hepatic fibrogenesis in alcoholic liver disease and is useful as a marker of fibrosis. Hepatology 1996. 24(6): 1399-1403.
3. Guechot J, Loria A, Serfaty L, Giral P, Giboudeau J, Poupon R: Serum hyaluronan as a marker of liver fibrosis in chronic viral hepatitis C: effect of alpha-interferon therapy. J Hepatol 1995, 22(1): 22-26.

【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

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