Instruction For Use

【Product Name】

HBsAg (eCLIA)

【Order Information】

REF NO.	Package Size
0320509911	50T
0320509912	100T
0320509915	2×50T
0320509916	2×100T

【Intended Use】

Immunoassay for the in vitro quantitative determination of hepatitis B surface antigen (HBsAg) in human serum and plasma. Determination of HBsAg is used for auxiliary diagnosis of suspected hepatitis B virus infection, for screening of blood donations and screening test to prevent transmission of hepatitis B virus.

【Summary】

Hepatitis B virus surface antigen (HBsAg) is a polypeptide with different molecular sizes, which is a component of the outer shell of hepatitis B virus (HBV) particles.[1,2] In addition to intact infectious particles, HBV patients also have a large number of non-infectious particles in their blood that consist only of a coat containing HBsAg.[3] HBsAg usually presents in the blood of people infected with HBV in one of two forms: shell proteins as virus particles surrounding them, or in the form of tubular and spherical particles with a diameter of 22 nm, which are free in the blood and are not infectious. HBsAg is the earliest detectable immune marker in serum after infection, and is usually detected within 2 to 12 weeks after infection and in the days or weeks before the onset of other clinical symptoms.[4] HBsAg determining site A is usually the main site for immune response detection and is ubiquitous in all HBsAg proteins. In addition, several subtypes of HBsAg can be identified within the "A" cluster, namely D, Y, W1-W4, R and Q.[5] The virus can express many different active HBsAg mutants (called "escape mutants"), while some mutants may cannot be detected by commercially available HBsAg testing reagents.[6-7] Hepatitis B virus surface antigen (HBsAg) marker is the most important and key indicator to determine whether hepatitis B virus is infected. It is usually used to assist in the diagnosis of patients suspected of HBV infection.[8]

HBsAg (eCLIA) was specifically developed to detect a multitude of these mutants. HBsAg is the first immunologic marker of HBV infection and is generally present some days or weeks before clinical symptoms begin to appear. Detection of HBsAg in human serum or plasma indicates the presence of acute or chronic HBV infection. HBsAg assays are used within the scope of diagnostic procedures to identify persons infected with HBV and prevent the transmission of the virus by blood and blood products. HBsAg assays can also be used to monitor the course of the disease and the efficacy of therapy in persons with acute or chronic HBV infections. In addition, HBsAg assays are recommended as part of prenatal care, in order to initiate suitable measures for preventing as far as possible the transmission of an HBV infection to the newborn child.

【Test Principle】

Sandwich principle. Total duration of assay:18 minutes.

50 µL of sample, biotinylated anti-HBsAg antibodies, and anti-HBsAg antibodies labeled with a 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 via a calibration curve which is instrument-specifically generated by 3-point calibration and a master curve provided via the reagent RIFD.

【Main Components】

The reagent pack consists of MB, RB, RA, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (50T)	Volume (100T)	Volume (2×50T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; ProClin300.	1×1.5 mL	1×3.0 mL	2×1.5 mL	2×3.0 mL
Reagent B (RB)	HBsAg-Antibody-Biotin, 0.3 mg/L; 0.1 M PBS; ProClin300.	1×2.5 mL	1×5.0 mL	2×2.5 mL	2×5.0 mL
Reagent A (RA)	HBsAg-Antibody-Ru, 0.36 mg/L; 0.1 M PBS, ProClin300.	1×2.5 mL	1×5.0 mL	2×2.5 mL	2×5.0 mL
HBsAg Calibrators	Negative calibrator, non reactive for HBsAg, 0.045M HEPES, ProClin300.	Cal 1: 1×1.5 mL			
	Positive calibrator, reactive for HBsAg, 0.05M HEPES, ProClin300.	Cal 2: 1×1.5 mL			
	Positive calibrator, reactive for HBsAg, 0.05M HEPES, ProClin300.	Cal 3: 1×1.5 mL			
HBsAg Control Materials (Optional)	Negative control, non reactive for HBsAg, 0.045M HEPES, ProClin300.	Level 1: 1×1.5 mL			
	Positive control, reactive for HBsAg, 0.05M HEPES, ProClin300.	Level 2: 1×1.5 mL			
	Positive control, reactive for HBsAg, 0.05M HEPES, ProClin300.	Level 3: 1×1.5 mL			

Traceability: This method can be traced to the NIBSC standard (code number: 12/226; WHO 3rd International Standard for HBsAg, subtype ayw1/adw2, genotype B4; IU/mL).

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 0320509906, Sample Diluent, 50 mL
- REF 679017, Enhanced Washing Buffer, 50 mL

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
- REF 0320509906, Sample Diluent, 50 mL

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, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105
- REF 0320509906, Sample Diluent, 50 mL

【Storage and Shelf Life】

Store at 2°C-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°C~8°C	18 months
Store at 2°C~8°C after opening	8 weeks
Store on the analyzers at 2°C~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°C~8°C	18 months
Store at 2°C~8°C after opening	8 weeks
Store on the analyzers at 18°C~28°C	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 Series: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use human serum samples or plasma samples collected with Li-heparin, Na-heparin, EDTA-K2, EDTA-K3, sodium citrate 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 with severe hemolysis are not in suggestion.

Samples can be stored for 8 hours at 18°C-28 °C, 7 days at 2°C-8 °C, 3 months at -20 °C ± 5 °C. The samples may be frozen 5 times.

Ensure the samples, calibrators and control materials are at 18°C-28 °C 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 of eCL series analyzer carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up HBsAg test according to the operational manual.

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

Recommended ambient temperature:10°C~30°C; 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 (RFID, 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 HBsAg control materials for quality control.

In addition, other suitable control material can be used.

Control material 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 IU/mL.

The analyzer automatically calculates the analyte concentration (IU/mL) based on the measurement of HBsAg Cal 1, HBsAg Cal 2 and HBsAg Cal 3.

Samples with a result < 0.05 IU/mL are non-reactive, these samples are considered negative for antibodies and do not need further testing.
Samples with a result ≥ 0.05 IU/mL are reactive, these samples should be retested in duplicate using the HBsAg (eCLIA).
All samples that are initially reactive must be centrifuged and retested in duplicate. If both tests are non-reactive, the samples are considered negative for HBsAg. If one or both tests are reactive, the samples are considered repeatedly reactive for HBsAg.
Repeatedly reactive samples must be investigated by a neutralizing confirmatory test. Samples which are confirmed by neutralization with human HBsAg-Antibody must be considered positive for HBsAg.

【Dilution】

Initial onboard dilution of 1:100 with Sample Diluent is mandatory for every sample by the analyzers. After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration. Samples with concentrations above the extended measuring range must be further diluted manually with Sample Diluent. In highly concentrated patient samples (>1300000 IU/mL), further manual dilution steps might be necessary to achieve results within the measuring range for pre-diluted samples. After manual dilution, multiply the result by the dilution factor chosen for the respective dilution step.
The dilution factor for dilution is 1:100. If a result is found within 5-13000 IU/mL for 100-fold diluted samples, no further dilution is necessary and endpoint result is achieved. If a result is found below the extended measuring range, the sample has to be run undiluted and should be found within 0.05-130 IU/mL. If a result is found > 13000 IU/mL for 100-fold diluted samples further manual dilution steps are recommended until result is found within the extended measuring range.

【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 ≤40 mg/dL, triglyceride ≤2000 mg/dL, biotin ≤30 ng/mL, human serum albumin ≤14 g/dL, hemoglobin ≤500 mg/dL, HAMA ≤100 ng/mL, the interference deviation of the determination results was within ±10%. Rheumatoid factors ≤1200 IU/mL, the relative recovery rate was between 90~110% and was not affected by the rheumatoid factor.
When the HBsAg concentration under 870000 IU/mL, the test results are not be affected by the high-dose hook effect.

Pharmaceutical substances

When the sample contains lamivudine (300 μg/mL), tenofovir (245 μg/mL), acetylcysteine (150 μg/mL), ampicilin sodium (1000 μg/mL), ascorbic acid (300 μg/mL), cyclosporine (5 μg/mL), cefoxitin (2500 μg/mL), levodopa (20 μg/mL), metronidazole (200 μg/mL), tetracycline (50 μg/mL), aspirin (1000 μg/mL), rifampin (60 μg/mL), acetaminophen (200 μg/mL), and ibuprofen (500 μg/mL), theophyline (100 μg/mL), entecavir (5 μg/mL), tibivudine (600 μg/mL) and adefovir (10 μg/mL), no interference with the assay was found. The interference deviation of the determination results was within ±10%.

Analytical specificity

Samples containing antibodies against HAV antibody, HCV antibody, HEV antibody, HIV-1/2 antibody, HSV-1 antibody, HSV-2 antibody, RV antibody, CMV antibody, EBV antibody, Toxo antibody, TP antibody, E.coli antibody, non-viral hepatitis, rheumatoid factors (RF) positive, anti-nuclear antibody (ANA) positive, human anti-mouse antibody (HAMA) positive are tested, and the HBsAg measured results are not greater than 0.05 IU/mL.

Wash procedure

In order to avoid interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium, it must performed the wash procedure by Enhanced Washing Buffer before each test. Please refer to the operator's manual.

Measuring range

Measuring range for pre-diluted samples:
The measurement range o f the kit is 5-13000 IU/mL for 100 fold diluted samples. Values below the measuring range are reported as < 5 IU/mL.Values above the measuring range are reported as > 13000 IU/mL.
When the samples are undiluted, the measurement range of the kit is 0.05-130 IU/mL. Values below the measuring range are reported as < 0.05 IU/mL.Values above the measuring range are reported as > 130 IU/mL.

【Analytical Performance】

Lower limit of measurement

Limit of Blank = 0.03 IU/mL
Limit of Detection = 0.05 IU/mL.

Accuracy

The relative deviation is within ±10%.

Measured values of control materials

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

Linearity

Within the range of 0.05 IU/mL~130 IU/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Precision

The precision of HBsAg are the Repeatability(%CV) ≤8% (Negative sample: SD≤0.01 IU/mL), the Within-Laboratory Precision(%CV) ≤10% (Negative sample: SD≤0.01 IU/mL), the Reproducibility(%CV) ≤10% (Negative sample: SD≤0.01 IU/mL).Precision was determined using reagents, samples in a protocol (EP5-A3) of the CLSI (Clinical and Laboratory Standards Institute). The following results:

Sample	Mean IU/mL	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human Sample, negative	0.025	2.15%	5.14%	4.23%
Human Sample, positive 1	1.050	1.43%	4.67%	2.64%
Sample, positive 2	94.57	1.16%	4.50%	2.41%

Homogeneity of calibrators and control materials

Within-bottle homogeneity:
Calibrator (Cal 1) and control material (Level 1): Standard deviation(SD)≤ 0.01 IU/mL;
Calibrators (Cal 2/Cal 3) and control materials (Level 2/Level 3): Coefficient of variation (CV)≤ 8%.

Between-bottle homogeneity:
Calibrator (Cal 1) and control material (Level 1): Standard deviation(SD)≤ 0.01 IU/mL;
Calibrators (Cal 2/Cal 3) and control materials (Level 2/Level 3): Coefficient of variation (CV)≤ 10%.

Diagnostic sensitivity

Samples from hepatitis B virus positive patients with different stages of the infection disease and unselected blood donors, hospitalised patients. The resulting sensitivity of confirmed positive samples was 100%. The 95% lower confidence limit was 99.45%.

Diagnostic specificity

Samples from unselected blood donors and hospitalised patients. The resulting specificity was 99.96%. The 95% lower confidence limit was 99.86%.

Method comparison

A comparison of the Lifotronic HBsAg assay (y) with the Roche Elecsys HBsAg II quant II. Method (x) using clinical samples gave the following correlations:
Number of samples measured: 699
Passing-Bablok regression: y=0.976x-0.0281
Pearson: r=0.997

【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.
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. 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】

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4. Bernard W, Anja Bayer, et al. Improved Detection of Hepatitis B Virus Surface Antigen by a New Rapid Automated Assay. Journal of Clinical Microbiology 1999;p:2639-2647.
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【Symbol 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		Contains Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limitation		This way up
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Manufacturer】

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