

Instruction for Use

【Product Name】

Anti-HBs (eCLIA)

【Order Information】

REF NO.	Package Size
0320513001	50T
0320513002	100T
0320513003	2×50T
0320513004	2×100T

【Intended Purpose】

Anti-HBs (eCLIA) is an immunoassay reagent for in vitro quantitative determination of antibody to hepatitis B virus surface antigen (Anti-HBs) in human serum and plasma. Determination of Anti-HBs is used for auxiliary diagnosis of hepatitis B virus infection.

Intended testing population: People who are suspected or infected with hepatitis B.

【Summary】

Anti-HBs is produced by human immune system after natural infection with hepatitis B virus (HBV) or vaccination against this virus, which is activated by the hepatitis B surface antigen (HBsAg)^[1]. The detection of Anti-HBs in the patient's serum indicates that the virus is gradually being cleared and the infection is trending towards recovery. As the concentration level of Anti-HBs increases, the immunity against HBV infection becomes stronger. The presence of Anti-HBs in individuals who have undergone vaccination indicates immunity to primary HBV infection. The concentration of Anti-HBs reaching 100 mIU/mL within 1 - 3 months after administration of the last vaccine dose is generally considered to have effective protection against infection in high-risk groups^[2]. Furthermore, quantitative determination of Anti-HBs is crucial for assessing the protection provided by vaccination and the need for booster doses^[3]. Quantitative detection of Anti-HBs levels can be also used for health management in post hepatitis-B liver-transplant recipients, with recommendations to maintain at least 100 - 500 IU/L^[4].

【Principle】

Sandwich principle. Total duration of assay: 18 minutes.

50 μL sample, complex formed by reaction with ruthenium-labeled HBsAg. The magnetic microparticle-antigen-antibody-antigen forms a sandwich complex after the addition of the magnetic microparticle-coated HBsAg. 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.

【Composition】

The reagent pack consists of MB, RA, RB, Calibrators and Control Materials (Optional). Different lot numbers of reagent components should not be used interchangeably.

Components	Ingredients	Content (50T)	Content (2×50T)	Content (100T)	Content (2×100T)
Magnetic Beads (MB)	Magnetic microparticle-coated HBsAg, 0.6 mg/mL; 0.05M PBS; 0.05% ProClin™ 300	1×1.5 mL	2×1.5 mL	1×3.0 mL	2×3.0 mL
Reagent B (RB)	0.05M PBS; 0.05% ProClin™ 300	1×3.3 mL	2×3.3 mL	1×6.5 mL	2×6.5 mL
Reagent A (RA)	Ruthenium-labeled HBsAg, 1.0 mg/L; 0.05 M PBS; 0.05% ProClin™ 300	1×3.3 mL	2×3.3 mL	1×6.5 mL	2×6.5 mL
Anti-HBs Calibrators	Anti-HBs; 0.05 M PBS; 0.05% ProClin™ 300	Cal 1: 1×1.0 mL Cal 2: 1×1.0 mL			
Anti-HBs Control Materials (Optional)	Anti-HBs; 0.05 M PBS; 0.05% ProClin™ 300	Level 1: 1×2.0 mL Level 2: 2×2.0 mL			

Traceability: This method has been standardized against the 2nd international standard substances for Anti-HBs (WHO 07/164).

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

➢ [REF] 0320509906, Sample Diluent, 50mL

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] 0320510002, Assay Cup/Assay Tip, 1×6×105

➢ [REF] 0320509906, Sample Diluent, 50mL

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

➢ [REF] 0320501801, Auffer, 2×2 L

➢ [REF] 0320501901, Buffer, 2×2 L

➢ [REF] 0320507503, PreClean, 2×2 L

➢ [REF] 0320510001, Assay Cup/Assay Tip, 6×6×105, or [REF] 0320510002,

Assay Cup/Assay Tip, 1×6×105

➢ [REF] 0320509906, Sample Diluent, 50mL

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【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.

Stability of the reagents	
Unopened store at 2°C-8°C	18 months
Store at 2°C-8°C after opening	8 weeks
Stored on the analyzer 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

【Specimen collection, handling and storage】

It is recommended to use human serum samples or plasma samples collected with heparin lithium, heparin sodium, EDTA-K2 and EDTA-K3 blood collection tubes. Samples should be collected in accordance with the standard operation of World Health Organization guidelines for blood collection. After the samples are completely coagulated, centrifugation should be performed to remove residual cellular substances. The samples should be free of air bubbles during testing. Lipid layer covering the samples after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 8 hours at 18°C-28°C, 14 days at 2°C-8°C, and 3 months at -15°C or lower than -15°C. The samples can be freeze-thawed only five times.

【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. Anti-HBs testing procedures should be 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°C humidity: ≤80.0%.

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 as follows :

- (1) When using a new lot of reagent;
- (2) When the same lot of reagents has been used on the analyzer for more than 28 days;
- (3) If the quality control result exceeds the defined limit;
- (4) When changing the buffers of different lot numbers.

Quality Control procedure

For quality control, use Anti-HBs Control Materials.

In addition, other suitable control material can be used.

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. Please make sure that the correct values are used.

Calculation

The system software can automatically calculate the analyte concentration, with the result in IU/L.

Specimen dilution

Samples with Anti-HBs concentrations above the detection range can be diluted with sample diluent. It is recommended to dilute the sample at 1:100 (automatic dilution by instrument or manual dilution), and the concentration after diluting the sample must be > 10 IU/L. If diluted by hand, the result should be multiplied by the dilution factor. If the instrument is automatically diluted, the instrument will automatically calculate the result.

【Interpretation of the results】

Result	Result message	Interpretation
< 10 IU/L	Non-reactive	Negative for Anti-HBs
≥ 10 IU/L	Reactive	Positive for Anti-HBs

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.

【Limitations】

1. The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
2. The measuring range of the kit is 2 ~ 1000 IU/L. Values below the lower limit of detection are reported as < 2 IU/L; Values above the upper limit of detection are reported as > 1000 IU/L.
3. When jaundice (bilirubin) ≤ 100 mg/dL, hemolysis (hemoglobin) ≤ 500



- the interference deviation of the measured results is within $\pm 10\%$. When rheumatoid factor ≤ 1200 IU/mL, the recovery was between 90% and 110%.
- When Lamivudine ≤ 30 mg/dL, Adefovir ≤ 1 mg/dL, Entecavir ≤ 1 mg/dL, Tenofovir ≤ 60 mg/mL, Telbivudine ≤ 24.5 mg/dL in the sample, the interference deviation of the measured results is within $\pm 10\%$.
 - There is no hook effect when the concentration of Anti-HBs reaches 200000 IU/L.

【Analytical Performance characteristics】

Lower limits of measurement

Limit of Blank = 0.5 IU/L

Limit of Detection= 2 IU/L

Limit of Quantitation = 5 IU/L

Accuracy

The accuracy control sample to standardized traceability is tested, and the relative deviation of its test results is within $\pm 10\%$.

Linearity

Within the range of 5 IU/L ~ 1000 IU/L, the correlation coefficient (r) of the kit should be ≥ 0.9900 .

Precision

The precision of Anti-HBs are the Repeatability (%CV) $\leq 6\%$, the Within-Laboratory Precision (%CV) $\leq 10\%$, the Reproducibility (%CV) $\leq 10\%$. Precision was determined using Anti-HBs reagent, samples in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute). The following results were obtained:

Sample	Mean (IU/L)	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human serum 1	5.123	3.28%	3.32%	3.45%
Human serum 2	31.57	2.35%	2.62%	2.67%
Human serum 3	107.2	2.87%	2.70%	3.08%

Measured values of Control Material

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

Homogeneity of calibrators and control materials

Within-bottle homogeneity: coefficient of variation (CV) $\leq 6\%$.

Between-bottle homogeneity: coefficient of variation (CV) $\leq 6\%$.

Analytical specificity

Samples containing potentially interfering substances or derived from high-risk groups were tested with the assay. The specimens containing Anti-HAV, Anti-HCV, Anti-HEV, Anti-HGV, Anti-HIV, TP, EBV, RV, CMV, HSV, Toxoplasma, E.coli and Yeast were tested, no cross reaction was observed.

Diagnostic Sensitivity

426 samples has been measured with the Lifotronic Anti-HBs assay that another commercially anti-HBs assay detected as positive. The sensitivity was 99.77%.

Diagnostic Specificity

523 samples has been measured with the Lifotronic Anti-HBs assay that another commercially anti-HBs assay detected as negative. The specificity was 99.62%.

Method comparison

A comparison of the Lifotronic Anti-HBs assay (y) with the Contrast reagent Method (x) were determined. The correlation was found by Passing-Bablok regression analysis:

Number of samples measured: 412

Linear regression: $Y=1.0027x+0.0745$ $r=0.9949$

The sample concentrations were between approx 5.0 and 1000 IU/L.

【Precautions and Warnings】

- When using this kit, the relevant operation precautions of the laboratory must be observed and it needs to be operated by a professional ;
- The test results of this kit can only be used as clinical reference, the patient's clinical evaluation should be based on the patient's clinical symptoms/signs, medical history, other laboratory test results and treatment response, etc.;
- Due to methodological reasons or antibody specificity, the results of the same sample tested with reagents of different manufacturers may be different. Therefore, results obtained with different kits should not be directly compared, so as to prevent wrong medical interpretation; It is recommended that the Laboratory Department indicate the characteristics of the reagents in the test report issued to the clinician. If the reagent type is changed during series monitoring, continuous testing should be conducted, and the results should be compared with the original reagent results in parallel to re-determine the baseline value;
- This product contains animal-derived substances, and may have potential biological risks. 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.

【Bibliography】

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【Symbols Explanation】

Symbol	Title	Symbol	Title
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Lot number		Contains sufficient for <n> tests
	Serial number		Temperature limit
	This way up		Authorized representative in the European Community
	Unique device identifier		Catalogue number
	Material code		Warning
	Date of manufacture		

【Manufacturer】

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【Version and Revision】

Version: 1.0

Issue Date:2024-09-02