Instruction for Use

【Product Name】

Anti-HBc (eCLIA)

【Order Information】

REF NO.	Package Size
0320513301	50T
0320513302	100T
0320513303	2×50T
0320513304	2×100T

【Intended Purpose】

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

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

【Summary】

Anti-HBc is produced by human T lymphocytes stimulated by the hepatitis B virus core antigen (HBcAg). HBcAg is the core component of the hepatitis B virus, consisting of 183 to 185 amino acids, and has high immunogenicity[1]. Generally, anti-HBc can be detected in the serum shortly after the appearance of HBsAg in hepatitis B virus (HBV) infection, and anti-HBc often persist for life[2]. Anti-HBc persist both in persons who have recovered from HBV infection and in those who develop HBsAg-carrier status[3]. The detection of anti-HBc can serve as an indicator of existing or past HBV infection. High levels of anti-HBc indicate an ongoing infection and are often present with HBsAg; low levels of anti-HBc suggest past infection and are commonly associated with anti-HBs[4].

【Principle】

The principle of competition. Total experimental duration: 27 minutes.

50 μL of the samples were pretreated with the reducing agent. After the addition of the biotin-labeled HBcAg, a complex was formed with Anti-HBc antibody in the sample. After the addition of ruthenium-labeled Anti-Hbc antibody and streptavidin coated with particles, an immune complex forms with unconjugated biotin-labeled HBcAg, which is bound to solid through the interaction of biotin and streptavidin.

After incubation, the reaction mixture was aspirated into the measurement unit, where the microparticles were magnetically trapped onto the electrode surface. Unbound material were then removed with system reagents. A voltage was applied to the electrode, followed by induced chemiluminescence emission measured by a photomultiplier tube. Results are determined by a calibration curve, which is specifically generated instruments by two-point calibration and the principal curve provided via RFID

【Composition】

The reagent pack consists of MB, RA, RB, RD, 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)	Streptavidin-coated microparticles, 0.75 mg/mL; 100 mM TB; 0.05% ProClin™ 300	1×1.8 mL	2×1.8 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Ruthenium-labeled Anti-HBc antibody, 1.0 mg/L; 0.1M PBS; 0.05% ProClin™ 300	1×2.8 mL	2×2.8 mL	1×5.5 mL	2×5.5 mL
Reagent A (RA)	Biotin-labeled HBcAg, 1.5 mg/L; 0.1M PBS; 0.05% ProClin™ 300	1×2.0 mL	2×2.0 mL	1×4.0 mL	2×4.0 mL
Reagent D (RD)	0.1M DTT; 0.05 M MES	1×1.5 mL	2×1.5 mL	1×3.0 mL	2×3.0 mL
Anti-HBc Calibrators	Anti-HBc; 0.1 M HEPES; 0.05% ProClin™ 300	Cal 1: 1×1.0 mL Cal 2: 1×1.0 mL			
Anti-HBc Control Materials (Optional)	Anti-HBc; 0.1 M HEPES; 0.05% ProClin™ 300	Level 1: 1×2.0 mL Level 2: 1×2.0 mL			

Traceability: This method has been standardized against the 1st international standard substances for Anti-HBc (WHO 95/522).

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 0320507501, 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 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.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
Stored on the analyzer 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 store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC 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 Word 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 24 hours at 18ºC~28ºC, 7 days at 2ºC~8ºC, and 5 months at -15ºC 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-HBc 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: 10~30ºC 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-HBc 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/mL.

Specimen dilution

Samples with Anti-HBc concentrations above the detection range can be diluted with sample diluent. It is recommended to dilute the sample at 1:10 (automatic dilution by instrument), and it is not recommended to dilute the sample with concentration below 2.5 IU/mL. If manually diluted, the result should be multiplied by the dilution factor, and the maximum dilution ratio should not exceed 1:40. If the instrument is automatically diluted, the instrument will automatically calculate the result.

【Interpretation of the results】

Result	Result message	Interpretation
< 0.5 IU/mL	Non-reactive	Negative for Anti-HBc
≥ 0.5 IU/mL	Reactive	Positive for Anti-HBc

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 0.18 ~ 4.0 IU/mL. Values below the lower limit of detection are reported as < 0.18 IU/mL; Values above the upper limit of detection are reported as > 4.0 IU/mL.

mg/dL, lipid (triglyceride) ≤ 1500 mg/dL, biotin ≤ 100 ng/mL in the sample, the interference deviation of the measured results is within ± 10%. When rheumatoid factor ≤ 1500 IU/mL, the recovery was between 90% and 110%.

4. When Lamivudine ≤ 300 mg/L, Adefovir ≤ 8 mg/L, Entecavir ≤ 0.8 mg/L, Tenofovir ≤ 245 mg/L, Telbivudine ≤ 480 mg/L in the sample, the interference deviation of the measured results is within ± 10%.

【Analytical Performance charateristics】

Lower limits of measurement

Limit of Blank = 0.10 IU/mL
Limit of Detection= 0.18 IU/mL
Limit of Quantitation = 0.25 IU/mL

Accuracy

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

Linearity

Within the range of 0.25 IU/mL ~ 4.0 IU/mL, the correlation coefficient (r) of the kit should be ≥ 0.9900.

Precision

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

Sample	Mean (IU/mL)	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human serum 1	0.405	3.12%	3.88%	3.25%
Human serum 2	1.059	2.97%	2.92%	2.97%
Human serum 3	2.629	2.81%	2.84%	2.83%

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) ≤ 6 %.
Between-bottle homogeneity: coefficient of variation (CV) ≤ 6 %.

Analytical specificity

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

Diagnostic Sensitivity

Samples from Anti-HBc positive patients were evaluated. The resulting sensitivity of confirmed positive samples was 100%. The 95% lower confidence limit was 98.60%.

Diagnostic Specificity

Samples from unselected blood donors and hospitalised patients were evaluated. The resulting specificity was 99.80%. The 95% lower confidence limit was 99.76%.

Method comparison

A comparison of the Lifotronic Anti-HBc assay (y) with the Contrast reagent Method (x) were determined. The correlation was found by Passing-Bablok regression analysis:
Number of samples measured: 408
Linear regression: Y=0.9896 x+0.0021 r = 0.9848
The sample concentrations were between approx 0.25 and 4.0 IU/mL.

【Precautions and Warnings】

1. When using this kit, the relevant operation precautions of the laboratory must be observed and it needs to be operated by a professional ;
2. 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.;
3. 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;
4. 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;
5. 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;
6. 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】

1. Eren E, Watts NR, Dearborn AD, et al. Structures of Hepatitis B Virus Core- and e-Antigen Immune Complexes Suggest Multi-point Inhibition. Structure. 2016 Oct 2;24(10):1814-1826.
2. Shi Y, Wang Z, Ge S, et al. Hepatitis B Core Antibody Level: A Surrogate Marker for Host Antiviral Immunity in Chronic Hepatitis B Virus Infections. Viruses. 2023 May 3;15(5):1111.
3. Wang Q, Klenerman P, Semmo N. Significance of anti-HBc alone serological status in clinical practice. Lancet Gastroenterol Hepatol. 2017 Feb;2(2):123-134.
4. Song LW, Liu PG, Liu CJ, et al. Quantitative hepatitis B core antibody levels in the natural history of hepatitis B virus infection. Clin Microbiol Infect. 2015 Feb;21(2):197-203.

【Symbols Explanation】

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

【Manufacturer】

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