Instruction for Use

【Product Name】

HBeAg (eCLIA)

【Order Information】

REF NO.	Package Size
0320513101	50T
0320513102	100T
0320513103	2×50T
0320513104	2×100T

【Intended Purpose】

HBeAg (eCLIA) is an immunoassay reagent for in vitro quantitative determination of hepatitis B virus e antigen (HBeAg) in human serum and plasma. Determination of HBeAg is used for auxiliary diagnosis of hepatitis B virus infection.

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

【Summary】

HBeAg is a soluble protein produced by the cleavage of the pre-core protein encoded by the hepatitis B virus (HBV) pre-C/C gene[1]. The detection of HBeAg is generally associated with the presence of large quantities of virus as it is a surrogate of viral Replication. HBeAg can be detected in serum shortly after HBsAg during acute HBV infections and usually disappears before HBsAg, when alanine aminotransferase(ALT) levels peak, followed by the presence of the corresponding antibody (anti - HBe)[2]. In the case of chronic HBV infection, the hepatitis B virus e antigen may coexist with HBsAg[3]. The seroconversion from HBeAg to anti-HBe indicates the end of active viral replication, and the disease transitions from an immunologically active phase to an inactive carrier state[4].Quantitative detection of HBeAg can be used to monitor the effectiveness of antiviral treatment in HBV patients. The level of HBeAg in the serum can reflect the prognosis of the patient. Real-time monitoring of the reduction level and extent of HBeAg during treatment can more accurately predict the efficacy of antiviral drugs, which helps guide clinicians to develop individualized treatment plans for different patients.

【Principle】

Sandwich principle. Total duration of assay: 18 minutes.

25 μL sample, biotin-labeled HBeAg antibody and ruthenium-labeled HBeAg antibody 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 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)	Streptavidin-coated microparticles, 0.75 mg/mL; 100 mM PBS; 0.05% ProClin™ 300	1×1.8 mL	2×1.8 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Biotin-labeled HBeAg antibody, 0.4 mg/L; 50mM Tris; 0.05% ProClin™ 300	1×3.0 mL	2×3.0 mL	1×6.0 mL	2×6.0 mL
Reagent A (RA)	Ruthenium-labeled HBeAg antibody, 0.1 mg/L ; 50mM Tris; 0.05% ProClin™ 300	1×3.0 mL	2×3.0 mL	1×6.0 mL	2×6.0 mL
HBeAg Calibrators	HBeAg; 50m M PBS; 0.05% ProClin™ 300, liquid	Cal 1: 1×1.0 mL Cal 2: 1×1.0 mL			
HBeAg Control Materials (Optional)	HBeAg; 50mM PBS; 0.05% ProClin™ 300, liquid	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 HBeAg (WHO 129097/12).

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-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 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 8 hours at 18ºC~28ºC, 7 days at 2ºC~8ºC, and 3 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. HBeAg 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 HBeAg 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 PEI U/mL or IU/mL: 1.0 PEI U/mL≈1.0 IU/mL.

Specimen dilution

Samples with HBeAg concentrations above the detection range can be diluted with sample diluent. It is recommended that the maximum dilution ratio of the sample does not exceed 1:100 (automatic dilution by instrument or manual dilution). 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.

【Reference intervial】

Result	Result message	Interpretation
< 0.1 PEI U/mL	Non-reactive	Negative for HBeAg
≥ 0.1 PEI U/mL	Reactive	Positive for HBeAg

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 0.05~200 PEI U/mL. Values below the lower limit of detection are reported as < 0.05 PEI U/mL; Values above the

upper limit of detection are reported as ">200 PEI U/mL".

3. When jaundice (bilirubin) ≤ 100 mg/dL, hemolysis (hemoglobin) ≤ 1600 mg/dL, lipid (triglyceride) ≤ 1500 mg/dL, total protein ≤ 10 g/dL, biotin ≤ 100 ng/mL in the sample, the interference deviation of the measured results is within ± 10%. When rheumatoid factor ≤ 2400 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%.
5. There is no hook effect when the concentration of HBeAg reaches 3000 PEI U/mL.

【Analytical Performance charateristics】

Lower limits of measurement

Limit of Blank = 0.01 PEI U/mL
Limit of Detection= 0.05 PEI U/mL
Limit of Quantitation = 0.05 PEI U/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 0.05 PEI U/mL~ 200 PEI U/mL, the correlation coefficient (r) of the kit should be ≥0.9900.

Precision

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

Sample	Mean (PEI U/mL)	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human serum 1	0.085	3.12%	3.43%	4.11%
Human serum 2	5.305	2.53%	2.69%	2.66%
Human serum 3	52.35	2.78%	2.95%	2.88%

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

453 samples has been measured with the Lifotronic HBeAg assay that another commercially HBeAg assay detected as positive. The sensitivity was 99.33%.

Diagnostic Specificity

835 samples has been measured with the Lifotronic HBeAg assay that another commercially HBeAg assay detected as negative. The specificity was 99.76%.

Method comparison

A comparison of the Lifotronic HBeAg assay (y) with the Contrast reagent Method (x) were determined. The correlation was found by Passing-Bablok regression analysis:
Number of samples measured: 407
Linear regression: Y=0.9919 x+0.1092 r = 0.9954
The sample concentrations were between approx 0.05 and 200 PEI U/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. Hong X, Luckenbaugh L, Mendenhall M, et al. Characterization of Hepatitis B Precore/Core-Related Antigens. J Virol. 2021 Jan 13;95(3):e01695-20.
2. de Almeida Pondé RA. Dynamic profile of the HBeAg-anti-HBe system in acute and chronic hepatitis B virus infection: A clinical-laboratory approach. Mol Biol Rep. 2021 Jan;48(1):843-854.
3. Moini M, Fung S. HBsAg Loss as a Treatment Endpoint for Chronic HBV Infection: HBV Cure. Viruses. 2022 Mar 22;14(4):657.
4. Nguyen MH, Wong G, Gane E, et al. Hepatitis B Virus: Advances in Prevention, Diagnosis, and Therapy. Clin Microbiol Rev. 2020 Feb 26;33(2):e00046-19.

【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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【Version and Revision】

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