Instruction For Use

【Product Name】

HIV Ag+Ab (eCLIA)

【Order Information】

REF NO.	Package Size
0320508809	50T
0320508814	2×50T
0320508810	100T
0320508815	2×100T

【Intended Use】

Immunoassay for the in vitro qualitative determination of HIV P24 antigen and antibodies to HIV-1 and HIV-2 in human serum and plasma. Determination of HIV Ag+Ab is used for auxiliary diagnosis of human immunodeficiency virus, for screening of blood donations and screening test to prevent transmission of human immunodeficiency virus.

【Summary】

HIV is a genetically related member of the Lentivirus genus of the Retroviridae family. Infections with lentiviruses typically show a chronic course of the disease, with a long period of clinical latency, persistent viral replication and involvement of the central nervous system. HIV can be transmitted by sexual contact, exposure to blood or blood products, and prenatal or perinatal infection of a fetus or newborn.

Human Immunodeficiency Virus (HIV) isolates are currently grouped into two types, HIV-type 1(HIV-1) and HIV-type 2 (HIV-2). The worldwide main agent of AIDS is HIV-1, while HIV-2 is restricted to some regions of Western and Centrral Africa. There are three major groups of HIV-1, designed as M (Major), O (Outlier), N (non-M/non-O) and the more recently identified group P[1]. Group M includes 9 subtypes, designed as A to K, which have spread with specific geographic distribution in the worldwide pandemic[2]. HIV-1 groups O and N, in contrast, are largely confined in restricted areas as Gabon, Cameroon and neighbouring countries[3]. Within subtypes A and F, at least 6 sub-subtypes (A1, A2, A3 and A4 and F1 and F2) can be distinguished. Occasionally, two viruses of different subtypes can infect the same cell and share their genetic material, thus resulting in new hybrid mosaic viruses. Many of these new strains are not replicating, but those able to be shed and trasmitted are known as “circulating recombinant forms” or CRFs. HIV-2 strains have been classified into at least five groups (A through E).

HIV P24 antigens are detectable in serum as early as 2-3 weeks after infection. Anti-HIV antibodies can be detected in blood specimen from around 4 weeks post infection. The Lifotronic reagent pack belongs to fourth-generation HIV screening assays, which can detect both antigens (P24) and antibodies against HIV-1 and HIV-2. These assays have been extensively evaluated and have proven to have high sensitivity and specificity, thus narrowing the window period and enabling the detection of acute and early HIV infection[4-6]. Furthermore, they have been proposed as a potential cost-effective alternative to nucleic acid amplification tests (NAATs) in HIV screening of populations with a high prevalence of HIV infection[7,8]. Finally, these assays have demonstrated a high sensitivity and specificity in the diagnosis of infections by genetically diverse viruses[9-11].

【Test Principle】

Sandwich principle. Total duration of assay: 27 minutes.

Pretreatment of 100 µL of sample with detergent agent. Biotinylated monoclonal anti-P24 antibodies/HIV-specific recombinant antigens and monoclonal anti-P24 antibodies/HIV-specific recombinant antigens with 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 automatically by the software via a calibration curve which is instrument specifically generated by 2-point calibration.

【Main Components】

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

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles 0.45mg/mL; 0.1M PBS, ProClin™300.	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
Reagent B (RB)	Biotinylated monoclonal anti-P24 antibodies/HIV-specific recombinant antigens 1.3 mg/L; 50 mM Tris, ProClin™300.	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
Reagent A (RA)	monoclonal anti-P24 antibodies/HIV-specific recombinant antigens with ruthenium 1.5 mg/L; 50 mM Tris, ProClin™300.	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
Reagent C (RC)	50 mM MES, ProClin™300.	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
HIV Ag+Ab Calibrators	Negative calibrator, non reactive for HIV (antigens and antibodies), 50 mM Tris, ProClin™300.	Cal 1: 1×1.5 mL			
	Positive calibrator, reactive for Anti-HIV-1 antibody, 50 mM Tris, ProClin™300.	Cal 2: 1×1.5 mL			
HIV Ag+Ab Controls (Optional)	Negative control, non reactive for HIV (antigens and antibodies), 50 mM Tris, ProClin™300.	Level 1: 2×1.5 mL			
	Positive control, reactive for Anti-HIV-1/HIV-2 antibodies, 50 mM Tris, ProClin™300.	Level 2: 2×1.5 mL			
	Positive control, reactive for HIV-1 P24 Antigen (E.colli), 50 mM Tris, ProClin™300.	Level 3: 2×1.5 mL			

Traceability: This method can be traced to HIV-1 P24 antigen, 1st International Reference Reagent 1992, NIBSC code: 90/636.

No internationally accepted standard for anti-HIV-1/HIV-2 Ab exist and can be

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 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T
- 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, o 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 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, o REF 0320510002, Assay Cup/Assay Tip, 1×6×105.

【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	12 weeks
Store on the analyzers at 2°C~15°C	8 weeks

The calibrators and controls are stable up to the stated expiration date.

Stability of the calibrators and controls	
Unopened store at 2°C~8°C	18 months
Store at 2°C~8°C after opening	12 weeks
Store on the analyzers at 18°C~28°C	8 hours

Store calibrators and controls upright in order to prevent the calibrators and controls 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 would better to be tested in 8 hours after collection, otherwise, should be stable for 7 days at 18°C-28°C, 4 weeks at 2°C-8°C or 3 months at -20°C±5°C. Freezing and thawing cycle is permitted 5 times.

Ensure the samples and calibrators are at 18°C-28°C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up HIV Ag+Ab test according to the operational manual.

Put the HIV Ag+Ab 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 (refer to instrument user manual).

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same reagent lot was used on the analyzer beyond 28 days;

(3) Quality control findings outside the defined limits;

(4) The Buffer of different lots are used.

Quality control

For quality control, use HIV Ag+Ab Controls.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least

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 analyzer automatically calculates the analyte concentration via a calibration curve which is instrument specifically generated by 2-point calibration.
The result of a sample is given either as reactive or non-reactive as well as in the form of COI.

【Interpretation of the results】

Samples with COI< 1.0 are considered non-reactive. These samples are considered negative for HIV-1 P24 antigen and anti-HIV-1/HIV-2 antibodies and do not need further testing.
Samples with COI ≥ 1.0 are considered initially reactive.
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 HIV-1 P24 antigen and anti-HIV-1/HIV-2 antibodies. If one or both tests are reactive, the samples are considered repeatedly reactive for HIV-1 P24 antigen and/or anti-HIV-1/HIV-2 antibodies.
Reactive samples must confirmed according to recommended confirmatory algorithms. Confirmatory tests include Western Blot and HIV RNA tests.

【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 ≤ 60 mg/dL, triglyceride ≤ 3000 mg/dL, biotin ≤ 30 ng/mL, hemoglobin ≤ 500 mg/dL, RF ≤ 1500 IU/mL, ANA ≤ 400 U/L, and HAMA ≤ 250 ng/mL, the interference deviation of the measured results is within ±10%.

Pharmaceutical substances

When the sample contains zidovudine (5.34 μg/mL), lamivudine (4.59 μg/mL), tenofovir (5.74 μg/mL), efavirenz (6.31 μg/mL), lopinavir (12.58 μg/mL), abacavir (5.73 μg/mL), tenofovir disoproxil fumarate (12.71 μg/mL), emtricitabine (4.95 μg/mL), nevirapine (5.33 μg/mL), rilpivirine (7.33 μg/mL), ritonavir (14.42 μg/mL), darunavir (10.95 μg/mL), raltegravir (8.89 μg/mL), and indinavir sulfate (14.24 μg/mL), no interference with the assay was found.
No false negative result due to high-dose hook effect was found.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
A negative test result does not completely rule out the possibility of an infection with HIV. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of HIV infection can occasionally yield negative findings. Yet unknown HIV variants can also lead to a negative HIV finding. The presence of HIV antigen or antibodies to HIV is not a diagnosis of AIDS.

【Analytical Performance】

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Limits and ranges

Antigen detection
Limit of Detection = 2 IU/mL.
The stated sensitivity was determined by reading off the HIV-1 P24 antigen concentration corresponding to the single of the cutoff value from standard curves obtained by serial dilutions of the HIV-1 P24 antigen-1st International Reference Reagent 1992, code: 90/636-in human HIV-negative serum.

Precision

Precision was determined using samples in a protocol (EP5-A3) of the CLSI. The following results were obtained:

Sample	Mean (COI)	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human Sample, negative	0.799	1.72%	4.38%	3.10%
Positive for anti-HIV-1 antibody	1.182	1.32%	4.55%	3.14%
Positive for anti-HIV-2 antibody	1.200	1.16%	4.70%	3.04%
Positive for HIV antigen	1.259	1.22%	4.57%	3.53%

Homogeneity of calibrators and control materials

Within-bottle homogeneity: The coefficient of variation (CV) ≤ 8.0%.
Between-bottle homogeneity: The coefficient of variation (CV) ≤ 10.0%.

Measured values of control materials

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

Analytical specificity

Samples containing HBsAg, Anti-HBs, Anti-HBe, Anti-HBc, Anti-HCV, Anti-TP, HSV-1 IgG, CMV IgG, EBV IgG, pregnant females, multiparous females, multiple myeloma, RF, ANA, HAMA were tested, no cross reactions were observed.

Diagnostic sensitivity

30 Seroconversion panels were tested, clinical sensitivity during seroconvresion represented the state of the art and all seroconvresion HIV samples were identified as positive.
570 from individuals clinically diagnosed with HIV infection and classified disease status were tested. The sensitivity of positive samples was confirmed to be 100%. The 95% lower confidence limit is 99.33%.

Group	A	Reactive
Anti-HIV-1 antibody positive samples	400	400
Anti-HIV group M positive samples	15	15
Anti-HIV group O positive samples	5	5
Anti-HIV-2 antibody positive samples	100	100
HIV-1 P24 antigen positive samples	50	50

Diagnostic specificity

In a group of 5000 unselected blood donors and 200 hospitalised patients, the clinical specificity was found 99.94%. The 95% lower confidence limit is 99.83%.

【Precaution and Warning】

1. The kit is only used for in vitro diagnostics.
2. Exercise the normal precautions required for handling all laboratory reagents.
3. All reagents and samples including specimen, reagents and calibrators should avoid foaming before and during test.
4. Safety data sheet available for professional user on request.
5. This product contains animal-sourced materials and may have potential biological risk. 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.
6. 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.
7. 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】

[1] Vallari A, Holzmayer V, Harris B, et al. Confirmation of putative HIV-1 group P in Cameroon[J]. Journal of virology, 2011, 85(3): 1403-1407.
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[3] Peeters M, Gueye A, Mboup S, et al. Geographical distribution of HIV-1 group O viruses in Africa[J]. AIDS (London, England), 1997, 11(4): 493-498.
[4] Sickinger E, Stieler M, Kaufman B, et al. Multicenter evaluation of a new, automated enzyme-linked immunoassay for detection of human immunodeficiency virus-specific antibodies and antigen[J]. Journal of Clinical Microbiology, 2004, 42(1): 21-29.
[5] Kwon J A, Yoon S Y, Lee C K, et al. Performance evaluation of three automated human immunodeficiency virus antigen–antibody combination immunoassays[J]. Journal of virological methods, 2006, 133(1): 20-26.
[6] Chavez P, Wesolowski L, Patel P, et al. Evaluation of the performance of the Abbott ARCHITECT HIV Ag/Ab combo assay[J]. Journal of Clinical Virology, 2011, 52: S51-S55.
[7] Long E F. HIV screening via fourth-generation immunoassay or nucleic acid amplification test in the United States: a cost-effectiveness analysis[J]. PloS one, 2011, 6(11): e27625.
[8] Karris M Y, Anderson C M, Morris S R, et al. Cost savings associated with testing of antibodies, antigens, and nucleic acids for diagnosis of acute HIV infection[J]. Journal of clinical microbiology, 2012, 50(6): 1874-1878.
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[10] Kfutwah A, Lemée V, Ngono H V, et al. Field evaluation of the Abbott Architect HIV Ag/Ab combo immunoassay[J]. Journal of Clinical Virology, 2013, 58: e70-e75.
[11] Rodgers M A, Vallari A S, Yamaguchi J, et al. ARCHITECT HIV combo Ag/Ab and realtime HIV-1 assays detect diverse HIV strains in clinical specimens[J]. AIDS research and human retroviruses, 2018, 34(3): 314-318.

【Symbols Explanation】

Symbol	Title	Symbol	Title
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Batch code		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Catalogue number	P/N	Material code
	Warning		Date of manufacture
	Unique device identifier		

【Manufacturer】

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【Version and Revision】

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