Performance on PRB109 anti-HIV-1 Low Titer Panel:

Sample\Kit	Erba Lisa® HIV Gen4	PCR	Chemi-system
PRB109-01	Positive	Positive	Positive
PRB109-02	Positive	Positive	Positive
PRB109-03	Positive	Positive	Positive
PRB109-04	Positive	Positive	Positive
PRB109-05	Positive	Positive	Positive
PRB109-06	Positive	Positive	Positive
PRB109-07	Positive	Positive	Positive
PRB109-08	Negative	Negative	Negative
PRB109-09	Positive	Positive	Positive
PRB109-10	Positive	Positive	Positive
PRB109-11	Positive	Positive	Positive
PRB109-12	Positive	Positive	Positive
PRB109-13	Positive	Positive	Positive
PRB109-14	Positive	Positive	Positive
PRB109-15	Positive	Positive	Positive
PRB109-16	Positive	Positive	Positive
PRB109-17	Positive	Positive	Positive
PRB109-18	Positive	Positive	Positive
PRB109-19	Positive	Positive	Positive
PRB109-20	Positive	Positive	Positive

Performance on NIBSC Panel:

Sample	Erba Lisa® HIV Gen4	Panel
Anti-HIV-1 Subtype A	Positive	1st International Reference Panel (02/210 HIV)
Anti-HIV-1 Subtype B	Positive	
Anti-HIV-1 Subtype C	Positive	
Anti-HIV-1 Subtype CRF01_AE	Positive	
Anti-HIV-2	Positive	
HIV-2 (Antibody) Monitor Sample	Positive	NIBSC 99/674

LIMITATIONS OF THE TEST

1. **Erba Lisa® HIV Gen4** is a screening test. All reactive samples should be further confirmed by supplemental assays such as Western blot, PCR etc.
2. A non-reactive test does not exclude the possibility of an HIV infection.
3. False positive results may occur due to non-specific binding of either the sample or the conjugate to the microwell.
4. The assay is only valid for human serum and plasma samples and not for other body fluids.
5. In case the kit is not stored properly or the test is not performed as per the recommended instructions, it may lead to erroneous results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

Transasia Bio-Medicals Ltd. products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Transasia Bio-Medicals Ltd. for which liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. Transasia Bio-Medicals Ltd. disclaims any and all responsibility for any injury or damage or legal implications which may be caused by the fault of the user or the buyer in accordance with the limitations and specifications herein. Due to continuous development, the manufacturer reserves the right to improve/change any specifications/components without prior information/notice to the buyer.

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For IVD Use Only

Code : 131790 Issue : 01, 30/09/2014

ISO 9001, ISO 13485
QUALITY SYSTEM CERTIFIED

TRELISAHIVGEN401091415

TRANSASIA BIO-MEDICALS LTD.
B-11, OIDC, Ringanwada, Daman - 396 210, India.

Erba Lisa® HIV Gen4

96 tests **Product Code: 131501**

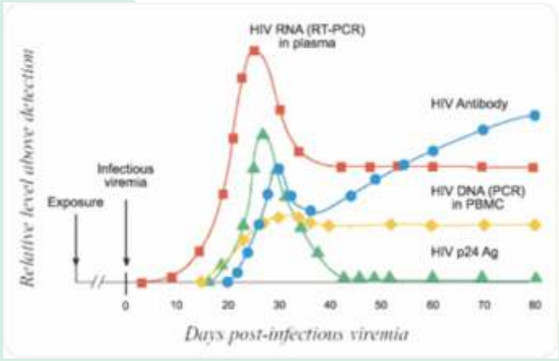
FOURTH GENERATION ENZYME LINKED IMMUNOSORBENT ASSAY KIT FOR DETECTION OF p24 ANTIGEN AND ANTIBODIES TO HUMAN IMMUNO-DEFICIENCY VIRUS IN HUMAN SERUM AND PLASMA

OVERVIEW

Acquired Immunodeficiency Syndrome or AIDS was first reported in the world in 1981. A couple of years later, the Human Immunodeficiency Virus (HIV) was discovered as the causative organism leading to AIDS. Since then, HIV/AIDS has been reported from all parts of the globe. The Human Immunodeficiency Virus (HIV) has changed the social, moral, economic and health fabric of the world in a short span. Today HIV/AIDS is the greatest health crisis faced by the global community. As per the 2008 UNAIDS report this pandemic has till date killed nearly 25 million people. It is estimated that more than 4.2 million people are living with HIV in the countries of the South-East Asia Region. India ranks as the country with the second largest number (2.4 million) of such people in the world and is next only to South Africa. Four countries (India, Thailand, Myanmar and Indonesia) are considered high-burden countries.
Antibodies to HIV are detectable within four to six weeks of infection by commonly employed tests and in virtually all infected individuals within six months. Once antibodies appear in the blood, they persist for the lifetime.

Diagnosis of HIV infection can be carried out by detecting either Antibodies to HIV or p24 HIV antigen or HIV nucleic acid (RNA/DNA). p24 antigen is an excellent marker of HIV expression and disease activity and can be used in the same fields of application as HIV RNA is used. p24 antigen testing is sensitive and specific in diagnosing pediatric and adult HIV infection, in predicting CD4+ T cell decline and clinical progression at early and late stage of infection, and suitable for antiretroviral treatment monitoring in both adults and children.

ELISA is the most widely used technique for the detection of antigen and antibodies to HIV.



INTENDED USE

Erba Lisa® HIV Gen4 is an in-vitro diagnostic kit for the early qualitative detection of antibodies for HIV-1, HIV-2 and p24 Antigen of HIV-1 in human serum or plasma. The kit is designed for use in blood banks to screen infected units as well as for clinical diagnostic laboratories.

PRINCIPLE

Erba Lisa® HIV Gen4 kit is a sandwich assay in which monoclonal antibodies to HIV-1 p24 and a mixture of recombinant antigens of HIV-1 and HIV-2 are coated onto the microwell plate. When human plasma or serum is added to the well, the anti-p24 antibodies bind to any p24 antigen in the sample while the bound HIV-1 and HIV-2 antigens will form a stable complex, if anti HIV-1 and / or HIV-2 antibodies are present. Followed by a wash step, conjugate containing a cocktail of monoclonal anti-p24-HRPO, recombinant HIV-1 and HIV-2 antigen-HRPO conjugate is added to the wells, followed by another washing step. Color reagent containing the substrate of HRPO is then added to the wells. Wells containing negative control or negative samples will remain colorless and blue color will develop in wells containing positive controls and test specimens containing p24 antigen and/ or antibodies to HIV. Upon addition of stop solution, blue color changes to yellow. The intensity of yellow color is directly proportional to the amount of bound p24 antigen/ anti-HIV antibody in the well.

KIT CONTENTS

Sr. No.	Reagents/Material	Presentation
1	***Coated Microwells*** – Breakaway microwells coated with a mixture of Recombinant HIV antigens gp120/41 and gp36 & monoclonal antibodies to HIV p24	8 wells x 12 strips
2	***Sample Diluent IG4*** – Ready to use, buffer with animal serum and detergent	4.0 mL
3	***Anti-HIV Negative Control*** – Ready to use, inactivated normal human serum	2.0 mL
4	***Anti-HIV Positive Control*** – Ready to use, inactivated human serum containing anti-HIV antibodies	2.0 mL
5	***HIV p24 Antigen Positive Control*** – Ready to use, recombinant HIV-1 p24 antigen	2.0 mL
6	***Washing Solution (20X conc.)***– Buffer containing surfactant. Dilute 1:20 with distilled water before use	30.0 mL
7	**Assay Buffer IG4** – Ready to use, protein-containing buffer to increase sensitivity and specificity of the conjugate.	6.0 mL
8	***HIV G4 Conjugate*** – Ready to use, HIV-1 recombinant gp120/41, HIV-2 recombinant gp36 and monoclonal antibodies to HIV-1 p24 antigen conjugated with HRP	6.0mL
9	***Color Reagent*** – Ready to use, 3,3',5,5'-Tetramethyl benzidine, Dimethyl sulfoxide, H_2O_2	6.0 mL
10	***Stopping Solution*** – Ready to use, phosphoric acid	12.0 mL
11	***Black Plate Cover*** – To avoid exposure to light during incubation	1
12	***Strip Sealers*** – Adhesive back strips for sealing wells during incubation in order to avoid evaporational loss	2
13	***Zip Lock Bag***	1

***Items 1 to 10 should be stored at 2-8°C.**

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MATERIALS REQUIRED BUT NOT PROVIDED

- Absorbent paper
- Disposable gloves and protective glasses
- Pipettes capable of delivering 25, 50 and 100 µL volumes
- Disposable tips
- Graduated cylinder
- Waste disposal container
- Timer
- ELISA reader (Lisa Scan™)
- Automatic / Semi-automated washing system (Lisa Wash™)
- Distilled / deionized water
- Sodium hypochlorite solution for disposal of waste
- 37°C Incubator

STORAGE

1. The shelf life of the kit is 18 months from the date of manufacturing when stored at 2-8°C.
   Do not FREEZE the components.
2. Immediately after use, return all reagents to 2-8°C.
3. The reconstituted wash buffer is stable for 2 months when stored at 2-8°C.
4. The unused microwell strips are suitable for use for 4 weeks after opening the foil pouch when stored at 2-8°C with desiccant in the zip lock bag.

PRECAUTIONS

- The test is for in-vitro diagnostic use and should be performed and interpreted by a competent or trained person only.
- DO NOT perform the test in case there is a cut or wound in the hands.
- Disposable gloves should be worn throughout the procedure.
- Before use, wait for all reagents to stabilize to room temperature.
- DO NOT use kit components beyond expiration date as indicated on the labels.
- DO NOT mix reagents between different lots as these are optimized for individual batch performance.
- All specimens and controls should be considered potentially infectious and discarded appropriately.
- Use a new tip for each controls and specimen.
- Use dedicated disposable tips to avoid microbial contamination of the reagents.
- Use clean glassware rinsed with distilled water.
- Use only distilled / deionized water to reconstitute the washing solution.
- DO NOT interchange caps of the reagents.
- Run the recommended number of positive and negative controls in each assay to ensure validity of the kit.
- Use Clear serum or plasma. Particulate matter should be removed by centrifugation.
- DO NOT allow the microplate wells to dry between the end of washing and the addition of the reagent.
- Incubation time should not vary by more than ± 2 minutes.
- After using required number of strips, rest of the strips along with activated silica gel should be kept in sealed condition into Zip lock bag .
- DO NOT expose color reagent to light, heat, metal ions or Peroxidase.
- Level the microwell strips in the holder before reading the results. Wipe the bottom of the wells with a clean tissue to remove any dust or moisture.
- Wash hands thoroughly with a suitable detergent, after the use of kit.
- In case of an accident, such as contact with eyes or contact of contaminated material with skin wounds or ingestion of contaminated material, etc. consult a physician immediately.
- Spills should be immediately decontaminated with a suitable disinfectant.
- Prior to disposal, all waste material should be collected and soaked in 5% sodium hypochlorite for at least 30 minutes.

SPECIMEN COLLECTION AND STORAGE

Erba LISA® HIV Gen4 is recommended to be used only for testing of human serum / plasma. Collect the specimen aseptically. Extract serum as soon as possible to avoid hemolysis. Samples containing aggregates must be centrifuged prior to use.

Fresh serum / plasma samples are preferred. Undiluted serum can be stored at 2-8°C for a week or frozen at -20°C until use. Frozen specimen should be completely thawed and centrifuged. The test should be performed on clear supernatant collected after centrifugation.
Heat inactivated, hemolyzed and icteric hyperlipemic samples may yield erroneous results.

RECONSTITUTION OF REAGENTS

Dilute washing solution 1:20 in distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage conditions. If so, use it after thawing at 37°C in a water bath.

ASSAY PROCEDURE

It is recommended that the assay procedure should be strictly adhered to, in order to obtain reliable results.

1) Bring all the reagents and test specimens to room temperature and shake well before use.
2) Define the sample / control distribution and identification plan. In each run, assign 3 wells for the HIV negative control (A1, B1, C1), 1 well for Anti-HIV Positive Control (D1) and 1 well for HIV p24 Antigen positive control (E1).
3) Break the number of required wells for a run. Wrap the balance unused wells tightly in zip-lock pouch with desiccant and return it to 2-8°C immediately.
4) Add 25 µL of the Sample Diluent IG4 to all the wells.
   Add 100 µL of the Anti-HIV Negative Control in wells A1, B1 and C1.
   Add 100 µL of the Anti-HIV Positive Control in well D1
   Add 100 µL of the HIV p24 Antigen Positive Control in well E1.
   Add 100 µL of the first sample in well F1, second sample in well G1 and so on.
   Mix well, cover the wells with strip sealers & black cover, incubate for 60 minutes at 37°C.
5) Remove the sealer. Discard / aspirate the contents of the well into the waste disposal container. Add a minimum of 350 µL of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing step 5 times more (Invert the plate and tap it on absorbent pad to remove the remaining washing solution).
6) Add 50µL of Assay Buffer IG4 & 50µL of HIV G4 Conjugate into all the wells.
   OR
   Mix Assay Buffer IG4 & HIV G4 Conjugate in equal volumes as per number of wells in a clean suspension tube just before the addition into the wells, Dispense 100µL in each well.

   For example: In case of 8 wells, mix 0.5ml of Assay Buffer IG4 & 0.5ml of HIV G4 Conjugate, add 100µL of mixture to each well.
   Mix well, cover the wells with strip sealers & black cover, incubate for 30 minutes at 37°C.
7) Repeat Step 5
8) Add 50µL of the Color reagent to all wells. Cover the plate with the black cover provided and allow the reaction to develop in the dark for 30 minutes at room temperature (20-30°C).
9) Add 100 µL Stopping solution to all wells. Homogenize. After the addition of the stopping solution the blue color of the substrate turns to yellow (for positive samples) or remains colorless (for negative samples).
10) Carefully wipe the plate bottom and read the Optical Density at 450 nm (using 620/630/650 nm as the reference filter) within 15 mins after pipetting of stop solution.

CALCULATION AND INTERPRETATION OF RESULTS

Negative Control: Absorbance values (O.D.) of the individual negative controls should be less than 0.125

Anti-HIV Positive Control: Absorbance value (O.D.) of the positive control should be greater than 1.0

HIV p24 Antigen Positive Control: Absorbance value (O.D.) of the positive control should be greater than 1.0
Calculate the mean of the measured absorbance values (O.D.) for the HIV Negative Control (Ncx)

Calculation of NCx:
Example:

NC	Absorbance
A1	0.044
B1	0.042
C1	0.046

NCx = (0.044+0.042+0.046) / 3 = 0.044
COV = 0.125 + 0.044 = 0.169

Calculation of the Cut-Off Value (COV)

COV = 0.125 + NCx

Note – If one reading for Anti-HIV Negative Control is out of range, NCx can be calculated using the other two readings.

Interpretation of the result:
Non-Reactive: Samples with an optical density less than the cut-off value are considered Non-Reactive.

Reactive: Samples with an optical density equal to or greater than the cut-off value are considered Initial Reactive.
These samples should be retested in duplicate. On retesting if the optical density of the duplicates is less than the cut-off value, the specimen is considered as Non-Reactive. If the retest result of the duplicates is found reactive, the specimen is considered Repeatedly Reactive.
Repeatedly Reactive specimens identified using **Erba LISA® HIV Gen4** must be further confirmed using other tests such as Western Blot or PCR.

Note:
The ELISA readers have a linear measuring range approximately 2.5 A. Beyond this range OD values are non-linear. Therefore many instruments programmed to show 'OUT' or 'OUT OF RANGE' indication, if the OD exceeds 2.5 or 3 or more, even after dilution of sample or control. Please note that this indicates a valid run provided the negative control value is <0.125 and does not interfere with assay results.

PERFORMANCE CHARACTERISTICS

Specimen Data	Total	Erba Lisa® HIV Gen4	Sensitivity/ Specificity
Total Number	555	555	
HIV Positive Panel 1	169	169	100%
HIV Negative Panel 1	386	386	100%
Total Number	**1305**	**1302**	
* HIV Positive Clinical Panel	**119**	**119**	**100%**
* HIV Negative Clinical Panel	**1186**	**1183**	**99.74%**

Overall sensitivity= 288/288= 100%
Overall specificity= 1569/1572= 99.8%

Analytical sensitivity of p24 Antigen = 8 IU/mL (as per NIBSC 90/636)

Performance on PRB978 HIV-1 Seroconversion Panel:
Erba Lisa® HIV Gen4 performance was found to be correlating with Chemiluminescence system on standard panel. **Erba Lisa® HIV Gen4** detects p24 Antigen 7 days post earliest RNA detection.

Sample\Kit	Days Since 1st Bleed (Fiebig Stage)	Erba Lisa® HIV Gen4	PCR	Chemi-system
PRB978-01	0 (NA)	Negative	Negative	Negative
PRB978-02	2 (NA)	Negative	Negative	Negative
PRB978-03	19 (NA)	Negative	Negative	Negative
PRB978-04	21 (NA)	Negative	Negative	Negative
PRB978-05	26 (I)	Negative	Positive	Negative
PRB978-06	28 (I)	Negative	Positive	Negative
PRB978-07	33 (II)	Positive	Positive	Positive