ASTAM DIAGNOSTICS

PRODUCT INSERT

REF HI3E

PRATHAM®

3rd GENERATION HIV 1 & HIV 2 ELISA

INTENDED USE

PRATHAM® 3rd Generation HIV1 & HIV2 ELISA is intended to be used for the detection of all subtypes (IgG, IgA, IgM) of antibodies to HIV 1 & HIV 2 viruses in human serum or plasma.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) causes Acquired Immune Deficiency Syndrome (AIDS), AIDS related complex (ARC) and pre-AIDS. Multiple subtypes have been documented through the use of genetic analysis. More than 90% of HIV-1 infections belong to HIV-1 group M3. HIV-2 is predominantly found in Africa and rarely elsewhere. HIV-1 and HIV-2 share a number of conserved sequences. However, serological cross reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample. HIV transmission occurs predominantly through exposure by sexual contact, exposure to blood including shared usage of contaminated needles and syringes, and contaminated blood products. Transmission may also occur from an infected mother to her unborn child during the prenatal period. When individuals are infected with HIV, their body produces antibodies to proteins in the HIV virus. The presence of these antibodies in various body fluids, including blood and oral fluid, are indicative of exposure to the HIV virus and can be used as an aid in the diagnosis of HIV infection. **3rd Generation HIV 1 & HIV 2 ELISA** is a solid phase Enzyme Linked Immunosorbent Assay (ELISA) which employs highly purified recombinant antigens representing most conserved antigenic segments of envelope glycoproteins, gp 120 and gp 41 of HIV-1 and gp 36 of HIV-2. The use of recombinant antigens both as capture and tracer reagent allows detection of all subtypes (IgG, IgA, IgM) of antibodies.

TEST PRINCIPAL

PRATHAM® 3rd Generation HIV1 & HIV2 ELISA Microwell strips are coated with recombinant antigens gp 120, gp41 and gp 36 representing both HIV-1 and HIV-2. Samples along with positive and negative controls are added in the coated wells and incubated. The wells are washed to remove unbound components and HIV1 and HIV2 recombinant antigens conjugated to Horse Reddish Peroxides are added. After a short incubation the wells are washed again and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450nm with an ELISA reader. The cut-off value is calculated by the given formula and absorbance of all the wells are compared with the cut-off value. Any sample having absorbance more than the cut-off value is considered reactive.

KIT COMPONENTS

Component	Description of Reagent	Presentation
Coated Microwells	96 Microwells (12x8) are coated with recombinant antigens representing both HIV 1 & HIV 2.	1
Positive Control	Inactivated and stabilized human serum reactive for HIV 1 with preservatives.	1x1.5 ml
Negative Control	Inactivated and stabilized human serum non-reactive for HIV-1 and HIV-2, HBsAG and HCV.	1x1.5 ml
Sample dilution buffer	Buffered solution containing stabilizing proteins and preservatives.	1x6 ml
Conjugate	Recombinant HIV 1 & HIV 2 HRP conjugate (21X). To be diluted 21 times with conjugate dilution buffer.	1x0.8 ml
Conjugate dilution buffer	Buffered solution containing proteins and preservative.	1x12 ml
Substrate	Solution containing Tetramethibenzidine (TMB) and hydrogen peroxide. Ready to use.	1x10 ml
Wash buffer	Buffer containing surfactants (40X). To be diluted 40 times with distilled or deionized water.	1x25 ml

Stop solution	Diluted sulphuric acid.	1x10 ml
Plate sealer	Adhesive paper sheet for covering plate on incubation time	2 Nos.
Pack insert	Instruction for use	1 Nos.

MATERIAL REQUIRED BUT NOT PROVIDED

Microplate reader capable of measuring absorbance at 450nm, Pipettes and Pipettes tips, Deionized or distilled water, 1X PBS, Automated microplate washer, ELISA Reader, Disinfectant, Timer, Gloves, Biohazard Waste Container etc.

STORAGE AND STABILITY

PRATHAM® 3rd Generation HIV 1 & HIV 2 ELISA kit is stable at 2-8ºC up to the expiry date printed on the label. Coated microwells should be used with in two months of opening the pouch. Any unused well (s) should be resealed with tape to prevent moisture absorption and stored at 2-8ºC for future use. In case the desiccant pouch changes color from blue to white, the strips should not be used. Diluted wash buffer should preferably be used with in the same day. However, excess wash buffer may be stored at 2-8ºC for 1 week.

SPECIMEN COLLECTION AND STORAGE

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard procedure.
3. Specimen should be free of particulate matter and microbial contamination.
4. Preferably use fresh sample. However, specimen can be stored refrigerated for 24 hours. For long storage, freeze at - 20ºC or below. Specimen should not be frozen and thawed repeatedly. Maximum of two freeze/thaw cycles are allowed.
5. Do not use heat inactivated specimen.
6. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
7. No not use turbid, lipaemic, haemolysed, clotted or contaminated specimen.

WARNING AND PRECAUTIONS

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Before use, allow reagents to reach room temperature (+18-30ºC).
- Carefully reconstitute or dilute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washings operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new pipette tip for each sample.
- Washing the microplate is a critical step in the procedure: Follw the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
- Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped and blotted on absorbent paper or towels to minimize residual wash buffer.
- Never use the same container to distribute conjugate and development solution.
- Check the pipettes and other equipment for accuracy and correct operations.
- Do not change the essay procedure.

Preparation of Reagents prior to use:

Wash Buffer Preparation: Dilute wash buffer 40 times for example add 5 ml concentrated buffer to 195 ml distilled or deionized water.

Conjugate Preparation: Dilute conjugate 21 times as shown below:

Reagent (Strip)	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate (µl)	50	100	150	200	250	300	350	400	450	500	550	600
Conjugate dilution buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Leave well A1 as substrate control.
6. Add 50µl sample dilution buffer in each well except A1.
7. Add 10µl controls and specimen in separate wells mix gently.
8. Apply plate sealer and incubate for 60 minutes at 37ºC.
9. Wash each well by filling approximately 350µl diluted wash buffer and aspirating/flicking off six times and blot dry.
10. Add 100µl diluted conjugate in each well except A1 and incubate for 30 minutes at 37ºC.
11. Wash six times as in step 9 and blot dry.
12. Add 100µl substrate in each well including A1 and incubate at room temperature away from light for 15 minutes.
13. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
14. Blank ELISA reader with well "A-1".
15. Read the absorbance at 450nm with 630nm or above as reference within 30 minutes of stopping the reaction.

Quality Control

Negative Control: The individual absorbance value of negative controls should be less than 0.1.
Positive Control: The individual absorbance value of positive controls should be more than 1.0.
Calculation of Cut of Vlue
The cut off value is calculated by adding 0.2 to average absorbance value of negative control.
The cut-off vale = 0.2 + NC(Avg)
Example

NC	**Absorbance**
A1	**0.022**
B1	**0.026**

Avg. of NC= (0.022+0.026)/2= 0.024
cov = 0.2+0.024=0.224
The cut-off value is calculated by adding 0.2 to average absorbance value of negative control.

INTERPRETATION OF RESULTS

1. Samples with absorbance value equal to or less than the cut-off value are considered non-reactive by 3rd Generation HIV 1 & HIV 2 ELISA kit and are considered negative for HIV 1 / 2 antibodies.
2. Samples with absorbance value greater than cut-off value are considered reactive by 3rd Generation HIV 1 & HIV 2 ELISA kit. The reactive samples should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative for antibodies to HIV 1 / 2. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
3. If a sample is repeatedly reactive the probability of antibodies to HIV 1 / 2 are high, specially with patients at high risk or high absorbance values. Such samples should be retested with supplemental tests such as western blot. Specimens that are repeatedly reactive in 3rd Generation HIV 1 & HIV 2 ELISA but not reactive in additional testing are considered indeterminate and a further sample after 3 to 6 months should be tested.

INTERNAL QUALITY CONTROL

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.

PERFORMANCE CHARACTERISTICS

The kit has been evaluated with the 20 known panel of HIV positive and 70 negative samples. Following is the in-house evaluation.

Description	Negative	Positive	Total
HIV known Negative Specimen	70	0	70
HIV known Positive Specimen	0	20	20

Assay Sensitivity : 100%
Assay Specificity : 100%

LIMITATION OF THE TEST

1. **PRATHAM® 3rd Generation HIV 1 & HIV 2 ELISA** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HIV infection. Reactive sample should be retested with confirmatory assays.
2. Absence of HIV antibody does not indicate that an individual is absolutely free of HIV infection.
3. Since various tests for HIV differ in their performance characteristics and antigen composition, their reactivity patterns may differ.
4. Testing of pooled samples is not recommended.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

DISPOSAL

Consider all test run with human specimen as potentially infectious and discard using standard biosafety practices.

DISCLAIMER:

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

The manufacturer and distributors of this product shall not be liable for any losses, liability, claims, coasts or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative in the use of this product.

REFERENCES

1. Gallo, R.C. J. AIDS, 1990,30: p.380.
2. Clavel, F., K. Mansinho, L. Montagnier et al., New Eng. J. Med. 1957, 316: p.1346-48
3. Gallo, R.C., S.Z. Salahuddin, M. Popovic et al., Science 1984, 224:p-500-2

SYMBOLS

Read instructions for use	Name of Manufacturer	For single use only
No. of test	Expiry Date of Kit.	Date of manufacturing of IVD Kit
IVD In-vitro diagnostic use	Keep away from Sunlight	REF Reference Catalogue Number
Storage Condition		

ASTAM DIAGNOSTICS

Manufactured By:

ASTAM DIAGNOSTICS PVT. LTD.

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