VANSCAN™

HIV 4th Gen.
Microwell ELISA

Enzyme immunoassay for the simultaneous detection of HIV p24 Antigen & Antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) & Type 2 (HIV-2) in Human Serum or Plasma

INTENDED USE:

HIV Gen. 4 Microwell ELISA is a fourth generation Immunoassay for the qualitative determination of Antibodies to HIV-1, (group O and M), HIV - 2 and p24 Antigen of HIV-1 in Human Serum or Plasma.

INTRODUCTION:

In 1984, (3 years after the first Acquired Immunodeficiency Syndrome report of a disease that was to become known as AIDS), researchers discovered the primary causative viral agent, the Human Immunodeficiency Virus Type 1 (HIV-1). Epidemilogically, it has been reported that HIV-1 has already been disseminated worldwide. In 1986 a second type of HIV, called HIV- 2 was isolated from AIDS patients principally in West Africa, several European countries and South America.

Knowledge on genetic variability of the HIV virus strains was acquired by sequencing the GAG, POL, and ENV genes of the representative strains of each subtype. The HIV-1 viruses are divided into 2 groups: the M group, including 9 sub-types (A to I) and the O group. The HIV-2 virus includes 5 sub-types.

HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and of the infection. The newly developed kit allows the simultaneous detection of anti HIV-1 (M and O groups), anti-HIV-2 antibodies and p24 antigens of HIV-1.

PRINCIPLE OF THE TEST:

Recombinant HIV-1 and HIV-2 antigens and antibodies are adsorbed onto the wells of the microwell plate. The wells are coated with recombinant HIV-1 gp41 antigen, recombinant HIV-1 group O gp 41 antigen, recombinant HIV-2 gp 36 antigen and monoclonal anti HIV-1 p24 antibody.

Serum or plasma samples are added to these wells. If antibodies to HIV-1/HIV-2 are present in the sample, they will form stable complexes with the HIV-1 and HIV-2 antigens on the plate. If p24 antigen is present in the sample it will bind with the monoclonal antibody bound to the solid phase. Biotinylated antibody conjugate is added which binds to specific HIV p24 antigen already bound on the well. In a subsequent step, second conjugate is added which in turn binds to specific antibody already bound on the well. Unbound conjugate is washed away and a solution containing 3,3',5',5'- tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound conjugate develop a blue colour which is converted to a yellow color when the reaction is stopped with sulphuric acid.

Each well and the colour is read at 450 nm. A reference wavelength of 630 nm is recommended.

KIT CONTENTS:

1. **HIV Ag & Ab Coated Microplate/Microstrips**
   One plate of 96 microwells coated with HIV Antigens and Monoclonal anti-HIV p24 antibody. Allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C. Once opened, Microwells should be used within one month.
2. **Sample Diluent**
   One bottle of buffered solution containing proteins, stabilizer, and preservative.
3. **Negative Control**
   One vial containing normal human serum with preservative. Negative Control has been tested and found to be negative for anti-HIV 1+2, HBsAg, Anti-HCV and Syphilis.
4. **HIV Ag Positive Control**
   One vial containing inactivated human serum containing HIV p24 Antigen in a buffer. Positive Control has been tested and found to be negative for anti-HIV 1+2, HBsAg, Anti-HCV and Syphilis.
5. **HIV Ab Positive Control**
   One vial containing inactivated anti-HIV positive human serum in a buffer. Positive Control has been tested and found to be negative for HBsAg, Anti-HCV and Syphilis.
6. **Wash Solution Concentrate (20x)**
   One bottle containing 20x Wash Solution, phosphate buffer saline with detergent. For preparation of Working Wash Solution refer to Step wise Procedure Card provided along with this Kit.
7. **Conjugate Concentrate (101x)**
   One vial containing mixture of streptavidin and HIV Antigens conjugated to HRP with protein stabilizers and preservatives. For preparation of Working Conjugate Solution refer to Step wise Procedure Card provided along with this Kit.
8. **Conjugate Diluent**
   One bottle containing solution consisting of buffer, protein and preservatives.
9. **TMB Substrate (101x):**
   One vial containing 3,3',5',5'- Tetramethyl benzidine - TMB, (101x) contraining Dimethyl Sulfoxide (DMSO) as solvent.
10. **Substrate Buffer:**
    One bottle containing Substrate Buffer Containing Hydrogen Peroxide (HO) and Stabilizers. For Preparation of Working TMB Substrate solution, refer to the preparation of Working Reagents.
11. **Stop Solution**
    One bottle containing colourless solution of diluted acid and stabilizer.
12. **Microstrip Covers**
    Sheets to cover micro strip during incubation.
13. **Package Insert**
14. **Procedure card**

MATERIAL REQUIRED BUT NOT PROVIDED:

(A) Distilled or deionized water, (B) Graduated Cylinder for reagents dilutions, (C) Micropipettes, (D) Paper towels or absorbent paper, (E) Timer, (F) Incubator, maintained at 37±1°C, (G) ELISA Reader, (H) ELISA Washer, (I) Sodium hypochlorite solution (free available chlorine 50-500mg/dl) and J) Disposable gloves.

SPECIMEN TRANSPORT AND STORAGE

Store the samples at 2-8°C. Samples not required for assay within 7days should be stored frozen (-15 °C or colder). Avoid multiple freeze-thaw cycles. After thawing ensure samples are thoroughly mixed before testing.

PRECAUTIONS

1. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% Sodium Hypochlorite before work is continued. Sodium Hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed off as potentially Bio-Hazardous waste. Do not autoclave materials containing's Sodium Hypochlorite.
2. Do not pipette by mouth. Wear disposable gloves and eye protection while handlings specimens and performing the assay. Wash hands thoroughly thereafter.
3. Sulphuric acid used in Stop Solution is corrosive and should be handled with appropriate care. If it comes into contact with skin or

eyes, wash thoroughly with water. If any of the reagents come into contact with skin or eyes wash the area extensively with water.

4. Do not use the reagents beyond the stated expiry date.
5. Do not modify the test procedure or substitute reagents from other manufactures or other lots unless the reagents are stipulated as interchangeable.
6. Do not reduce any of the recommended incubation times.
7. Allow the reagents and samples to come to 18 to 30°C before use.Immediately after use, return reagents to the recommended storage temperature.
8. Do not allow wells to become dry during the assay procedure.
9. Do not cross-contaminate reagents. It is recommended to use dedicated separate pipettes for dispensing the Substrate Solution and Conjugate.
10. Ensure that the bottom of the plate is clean and dry and that no bubbles are presents on the surface of the liquid before reading the plate.

PREPARATION OF TEST:

1. Bring all the reagents to room temperature 30 minutes before use.
2. Take the required number of strips from the sealed HIV Ag and Ab coated plate. The remaining strips must be kept at 2-8°C with the silica gel after proper sealing of the pouch.

TEST PROCEDURE:

1. Take the required number of strips and fix them on to the plate.
2. Pipette 50 µl of Sample Diluent into each plate well except Blank well A1 and pipette 50 µl of Negative Control into each well from B1 to D1 and 50 µl of Ag. & Ab. Positive Control into wells E1 & F1 respectively, and then, pipette 50 µl of each sample into the remaining wells.
3. Incubate at 37±1° C for 30 min. after covering the plate with Microstrip Covers.
4. Before the last 5-10 minutes of the 1st incubation, make a 1:100 dilution of the Conjugate with Conjugate Diluent. Refer to the Step Wise Procedure Card provided along with this kit.
5. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution. Refer to the Step Wise Procedure Card provided along with this kit.
6. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100µl of prepared Working Conjugate Solution into each well except Blank well A1.
7. Incubate the plate at 37±1°C for 30 minutes after sealing it with the Microstrip Covers.
8. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution.
9. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100 µl of working Substrate Solution into each well including the Blank well A1. Refer to the Step Wise Procedure Card provided along with this kit.
10. Incubate it at controlled room temperature (21-25°C) for 30 minutes.
11. Pipette 50µl of Stop Solution into each well including Blank well A1.
12. Read the absorbance at 450nm (reference wavelength at 630nm) within 15 minutes after pipetting the Stop Solution.
14. Pipette 50 µl of Stop Solution to each well including Blank well(A1).
15. Read the absorbance at 450 nm (reference wavelength at 630nm) against air within 15 minutes after pipetting the Stop Solution.

QULITY CONTROL

Results of an assay are valid if the following criteria for the Controls are met:-

a) **Absorbance of Blank** should be **<0.050.**

b) **Mean Absorbance of Negative Controls** should be **< 0.100**. If absorbance of Negative Control comes in negative. eg. - 0.003 it should be considered as zero.

c) **Mean Absorbance of Positive Controls** should be **> 0.500.**

If the results are outside the above ranges, the test should be conducted again.

CUT OFF VALUE:

Calculations of the Cut off Value (COV) :

(a) Calculate the Negative Control mean ($NC\bar{x}$)

e.g.) Negative Control 1 absorbance = 0.009
Negative Control 2 absorbance = 0.010
Negative Control 3 absorbance = 0.011
Negative control mean ($NC\bar{x}$) =
(0.009+0.010+0.011)/3=0.010

(b) Calculate the Cut off Value (COV) :

Cut off Value (COV) = $NC\bar{x}$ +0.200 =0.010+0.200 =0.210

INTERPRETATION OF RESULTS

Reactive Results: - Samples with absorbances greater than or equal to the Cut off Value are considered Reactive in the assay.

Non-Reactive Results: - Samples with absorbances less than the Cut off Value are considered non-reactive in the assay.

NOTE: -If the samples are considered reactive, the test should be conducted two more times.

Gray zone samples: whose absorbance falls in between ±10% of cut-off.

In cases the re-tests show non-reactive, the samples are considered non-reactive, and on the other hand, if one of the re-tests shows reactive, the samples are considered reactive.

The samples considered reactive should be tested again by Western Blot etc. for final interpretation.

PERFORMANCE CHARACTERISTICS

Specificity	99.5%
Sensitivity	100%

BIBLIOGRAPHY

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