MaxLISA HIV 4.0 ELISA Test

(Enzyme Linked Immunosorbent Assay)

ORDERING INFORMATION

Ref. No.	**Pack Size**
AVELHIV4-96	96 Tests

1. INTENDED USE: MaxLISA HIV 4.0 (Ag+Ab) ELISA Test is an in vitro enzyme immunoassay for the qualitative determination of antibodies to HIV-1/HIV-2 and HIV-1 p24 antigen in human serum or plasma. It is intended for screening of blood donors or other individuals at risk for HIV-1 and HIV-2 infection and for clinical diagnostic testing. This test is not automated.

2. INTRODUCTION: Antigen can usually detect in acute phase and through symptomatic phase of AIDS and antibodies can be detected during the infection. It has been observed that the core protein of HIV-1 and HIV-2 show cross reactivity whereas envelope proteins are more specific and moreover antibodies against the envelope gene products can be found in almost all infected people. MaxLISA HIV 4.0 (Ag+Ab) ELISA Test has been developed and designed to be extremely sensitive and specific using recombinant proteins; gp41, C terminus of gp120 and gp36 representing the immunodominant regions of HIV1 & HIV-2 envelope gene structure respectively and HIV-1 p24 antibodies. Detection of p24 antigen also reduces window period.

3. PRINCIPLE: Current evidence specifies that Acquired Immunodeficiency Syndrome is caused by HIV-1 and HIV-2. The viruses are transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products or transmitted from an infected mother to her fetus or child during the prenatal period. Presence of antigen/antibodies to the virus in the serum of a patient indicates viral infection. Recombinant HIV-1 and HIV-2 antigens are adsorbed onto the wells of micro assay plate. Biotinylated sample diluent and 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 micro assay plate. The wells are washed to remove unbound components. If the antigen/antibody complex is present, the peroxidase conjugate will bind to antibody and remain in the well. After washing wells to remove unbound enzyme, substrate is added. Color will develop in wells containing antibody. No color will develop in wells having no antigen-antibody complex. An acid stopping solution is added to each well and the color is read on photometer at 450 nm and reference wavelength 620 nm is recommended.

4. KIT CONTENTS:
Store all components at 2-8°C when not in use

Material	**96 Tests**
HIV 1/2 Antigen & Anti HIV-1 p24 strips (1 x 96 well microplate)	8 wells X 12 strips, Micro wells coated Coated Micro HIV-1 & HIV-2 recombinant proteins and HIV- 1 p24 antibody packed in a pouch with Desiccant.
HIV antibody Positive control (1 x 1 ml)	Inactivated human serum, positive for HIV antibodies and non-reactive for HBsAg and HCV antibodies with preservative.
HIV antigen Positive control (1 x 1 ml)	Inactivated and anti-p24 antibody human serum, positive for HIV P24 antigen and non-reactive for HBsAg and HCV antibodies with Preservative.
Negative control (1 x 1 ml)	Inactivated and stabilized human serum reactive for HIVI, HIV2, HBsAg and HCV.
Sample diluent (1 x 3 ml)	Biotinylated agglutinating sera for p24 antigen.
Wash Buffer (20X) (1 x 25 ml)	Buffer containing surfactants
Conjugate diluent (1 x 20 ml)	Buffer solution containing stabilizing proteins and preservatives
Conjugate Concentrate (101X) (1 x 0.2ml)	Antigen-HRP/Streptavidin-HRP conjugate to be diluted with conjugate diluent.
TMB Substrate A (1 x 10 ml)	Buffer solution containing H2O2 with preservative.
TMB Substrate B (1 x 10 ml)	To be diluted in TMB diluent before use.
Stop solution (1 x 12 ml)	Ready to use, 0.IN Sulfuric acid
Pack insert	1 No.

5. MATERIALS REQUIRED BUT NOT PROVIDED
- Distilled or Deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves

6. SAMPLE COLLECTION, STORAGE & HANDLING
- Only human serum or plasma samples should be used for the test
- While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis
- Fresh serum/plasma samples are preferred.
- Serum and plasma (EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20 to -70°C)
- Avoid repeated freezing and thawing.
- Do not use sodium azide as preservative because it inactivates horseradish peroxidase
- Microbial contaminated and hemolyzed samples may give erroneous Results.

7 . PRECAUTIONS:
- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is strongly recommended while running the test.
- Before performing the test, read all the instructions carefully and follow each and every instruction to get the intended and accurate results.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Use the required volume of specimen while testing.
- Do not let the strips dry in between the steps.
- Follow GLP and biosafety guidelines for handling and disposal of potentially infective materials/expired kit/used kits.
- Carefully read and follow the assay procedure and storage instructions. Deviation will lead to erroneous results.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact wit heyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
- In case of performance changes or product malfunction, stop using the kit immediately and contact your local distributor.
- Controls and samples to be tested should be handled as potentially hazardous as they are capable of transmitting infection.

8. PREPARATION OF REAGENTS
Note: Before use, allow reagents and samples at room temperature (20-30°C).

8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ± 2-8°C

Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.
8.1.2. Negative control
8.1.3. HIV Antibody positive control
8.1.4. HIV Antigen positive control
8.1.4. Sample Diluent
8.1.6 Conjugate Diluent
8.1.7. Stop solution

8.2. Reagents to reconstitute:
8.2.1. Wash buffer (20X):

- **8.2.1. Wash buffer (20X):**
- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals Dissolve.
- Dilute 1:20 in distilled water to obtain the ready to use washing solution. Mix 25 ml of 20X wash buffer concentrate with 475 ml of distilled or deionized water. Working wash buffer is stable for 2 months when stored at 2-8°C.

8.2.2. Wash Procedure:
- Incomplete washing will adversely affect the test outcome.
- Aspirate the well contents completely into a waste container.
- Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another well.
- Aspirate completely and repeat the wash procedure for a total 5 times of washes.
- Automated washer if used should be well adjusted to fill each well completely.
- Tap plate on absorbent papers till no droplets appear on the paper

- **8.2.3. Preparation of working conjugate:**

Make a 1:101 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use. Determine the quantity of working conjugate solution to be prepared from table given below.
Mix solution thoroughly before use

Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Enzyme Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:

Mix TMB Substrate A and TMB Substrate B in 1:1 ratio to prepare working substrate buffer 5 to 10 minutes before use. Avoid exposure to light.

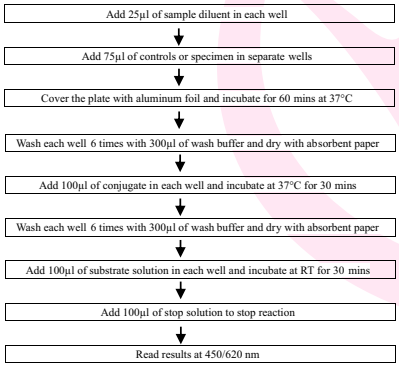
Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate A (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Substrate B (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

9. TEST PROCEDURE:

I. Bring all the reagents and specimen to room temperature before use.
II. Take the required number of strips and fix them to frame and immediately close the pouch. Prepare template in data sheet indicating the location of controls and Specimens.
III. Add 25µl of sample diluent in each well.(Mix Well before use)
IV. Add 75µl of negative control in each well no. A1, B1
V. Add 75µl of antibody positive control in well no C1
VI. Add 75µl of antigen positive control in well no D1
VII. Add 75µl of specimen in each well starting from E1.
VIII. Cover the plate with aluminum foil and incubate for 60 minutes at 37°C.
IX. Before 5 to 10 minutes of incubation, make a 1:101 dilution of conjugate with conjugate diluent.
X. After incubation, aspirate the contents from all the wells and wash each well 6 times with by filling approximately 300µl diluted wash buffer.
XI. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted conjugate into each well.
XII. Incubate the plate at 37°C for 30 minutes.
XIII. Before 5 to 10 minutes of incubation, make a 1:1 dilution of substrate A with substrate B.
XIV. Aspirate and wash as described in step no.10.
XV. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then Dispense 100µl of prepared diluted substrate into each well and incubate at room temperature for 30 minutes.
XVI. Add 100µl of stop solution each well.
XVII. Read absorbance at 450/620nm within 10 minutes in ELISA READER

10. FLOW CHART FOR TEST RUN CRITERIA:



11. Calculation of the cutoff value

A. Negative control means (NCx)

Absorbance of Negative control (NC)	
NC1	0.090
NC2	0.082
NC Mean (NCx)	(0.090+0.082)/2 = 0.086

B. Cut off value: NCx+0.2 = 0.086 + 0.2 = 0.286

C. Quality Control:

Results of an assay are valid if the following criteria for the controls are met:
PC must be ≥0.500.
NC must be ≤0.200

D. Abbreviations:

NC - Absorbance of Negative control
NCx - Mean Negative control
PC - Absorbance of the Positive control

12. INTERPRETATION OF RESULTS:

- Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for anti-HIV.
- Test specimens with absorbance value greater than or equal to the cut off value are reactive for HIV

13. LIMITATIONS:

- MaxLISA HIV 4.0 (Ag+Ab) ELISA Test assay is designed for testing antibodies against HIV-1 and/or HIV-2 in human serum and plasma.
- If possible, use fresh serum or plasma samples. Sample degradation as well as multiple freeze-thaw cycles may cause erroneous results.
- Do not use heat-inactivated samples.
- The user of this kit is advised to carefully read and understand the instructions.
- In establishing infection of HIV-1/HIV-2, in evaluating patients with AIDS symptoms, MaxLISA HIV 4.0 (Ag+Ab) ELISA Test testing alone cannot be used to diagnose AIDS even if antibodies against HIV are present in human serum or plasma.
- This is only screening test. All samples detected reactive must be confirmed by using Western blot (WB), and other confirmatory test

14. PERFORMANCE CHARACTERISTICS (Clinical Sensitivity, Specificity and Accuracy)

The MaxLISA HIV 4.0 (Ag+Ab) ELISA was evaluated in a multi-center field study, a blood donation center as well as an in-house clinical study. The multi-center study included 1259 specimens & HIV Performance Panel that was purchased from a commercial source. The MaxLISA HIV 4.0 (Ag+Ab) ELISA was compared to leading commercial ELISA HIV tests. Out of the1259 total specimens, 54 were found positive for HIV 1 and 5 specimens were found Positive For HIV 2 and 1200 Specimens were found negative by ELISA. The MaxLISA HIV 4.0 (Ag+Ab) ELISA showed 100 % relative sensitivity, and 99.8 % relative specificity compared to ELISA.

MaxLISA HIV 4.0 (Ag+Ab) ELISA vs HIV Rapid Test

	Observation				
	MaxLINE HIV 1 & 2 Triline		Commercial HIV ELISA		Total Results
Type of Specimen	Positive	Negative	Positive	Negative	
True Positive HIV 1	54	0	54	0	54
True Positive HIV 2	05	0	05	0	05
True Negative HIV1&2	2	1198	0	1200	1200

Sensitivity: 100% Specificity: 99.8 %

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of four specimens: a negative, a low positive, medium positive and a high positive. The negative, low positive, medium positive

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same four specimens: a negative, a low positive, medium positive and a high positive. Three different lots of the HIV 1 2 Tri-line Human Immunodeficiency Virus Rapid Test Device (Serum/Plasma/Whole Blood) have been tested using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified 100% of the time.

15. INTERFERING SUBSTANCES

The following potentially interfering substances were added to HIV 1 & HIV 2 negative and positive specimens.

Acetaminophen:	20 mg/dL	Caffeine:	20 mg/dL
Acetylsalicylic Acid:	20 mg/dL	Gentisic Acid:	20 mg/dL
Ascorbic Acid:	2g/dL	Albumin:	2 g/dL
Creatin:	200 mg/dL	Hemoglobin	1.1 mg/dL
Bilirubin:	1g/dL	Oxalic Acid:	600mg/dL

None of the substances at the concentration tested interfered in the assay.

16. REFERENCES:

- Centers for Disease Control, Update on Acquired Immune Deficiency Syndrome (AIDS) MMWR 1982; 31: 507-508.
- Constantine NT van der Groen G, Belsey EM, Tamashiro H. Sensitivity of HIV-antibody assays determined by seroconversion panels. AIDS, 1994; 8: 1715-1720.

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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