



HIV-1/2 Ab ELISA Kit

Catalog Number KA0292

96 assays

Version: 16

Intended for research use only

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Introduction

Intended Use

The HIV-1/2 Ab ELISA Kit is an enzyme immunoassay for qualitative determination of antibodies to HIV-1 and HIV-2 in human serum or plasma.

Background

Acquired immune deficiency syndrome (AIDS) was first recognized and described in 1981. It is caused by virus, which are transmitted by sexual contact, by sharing contaminated needles and syringes, by contaminated blood products, or transmitted from an infected mother to her fetus or child during the prenatal period. Public education of AIDS prevention plus HIV antibodies screening on donor blood can prevent wide spreading of AIDS infection.

The disease is considered to involve at least two different Human Immunodeficiency Viruses (HIV-1 and HIV-2). Evidence of widespread HIV-1 infection has been reported from almost every country in the world. HIV-2 infection has been found in only a few people in Europe and countries in West Africa. HIV-1 and HIV-2 have a substantial degree of immunological homology among their core proteins but their envelope glycoproteins show considerable differences. To ensure satisfactory detection of antibodies to both types of viruses, it is necessary that epitopes from envelope proteins of both viruses is used in screening assay.

Principle of the Assay

The HIV-1/2 Ab ELISA Kit adopts the "direct sandwich principle" as the basis for the assay to detect antibodies to HIV-1 and/or HIV-2.

The HIV-1/2 Ab ELISA Kit is a sandwich enzyme immunoassay which employs recombinant HIV-1 and HIV-2 antigens for the detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. These rDNA antigens which are reactive with the predominant antibodies to HIV-1 and HIV-2, constitute the solid-phase antigenic absorbent. When human serum or plasma is added to the well, the HIV-1/2 antigens and Anti-HIV 1/2 will form complexes on the well if Anti-HIV 1/2 is present in the specimen. After washing, the well is filled with a solution containing conjugate of rDNA HIV-1/2 antigens and horseradish peroxidase (HRPO), allowing the formation of (HIV-1/2) • (Anti-HIV-1/2) • (HIV-1/2 • HRPO) complex. After washing out the unbound conjugate, TMB solution (3,3',5,5'-tetramethylbenzidine) is added for color development. The absorbance of the color development is a measurement of the Anti-HIV1 and/or Anti-HIV2 content in the sample.

General Information

Materials Supplied

List of component

Component	Description	Amount
HIV 1+2 Antigens Plate	96-well microtiter plate coated with rDNA HIV-1 env (gp120/41) and HIV-2 env peptides (gp 105/gp36) antigens.	1 plate
Conc. HIV Antigen Conjugate	rDNA HIV-1 and HIV-2 antigens conjugated with horseradish peroxidase (HRPO), diluted in Tris-buffer with bovine serum. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle 0.3 mL
Anti-HIV1 Positive Control	Inactivated rabbit serum positive for anti-HIV1. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle 1.5 mL
Anti-HIV2 Positive Control	Inactivated rabbit serum positive for anti-HIV-2. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle 1.5 mL
HIV Negative Control	Normal human serum non-reactive for HBsAg, anti-HCV and for anti-HIV-1 and 2. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle 1.5 mL
Conjugate Diluent D	Tris-buffer contains bovine serum. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle 20 mL
TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle 12 mL
TMB Substrate Solution B	A Citric buffer contains H ₂ O ₂ .	1 bottle 12 mL
2 N Sulfuric Acid	2 N Sulfuric Acid	1 bottle 12 mL
Washing Solution D (20X) Concentrate	A 20X concentrated Phosphate buffer with Tween-20.	1 bottle 110 mL

Accessories: (provided as needed)

Component	Amount
Adhesive slips	X1
Black cover	X1

Storage Instruction

- ✓ The kit must be stored at 2 to 8°C. Do not freeze.
- ✓ Strips of the plate should be used within 1 month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag, taped the opening tightly and stored at 2 to 8°C.
- ✓ Return the reagents to 2 to 8°C immediately after use.
- ✓ Washing Solution D (20X) should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in 37°C water bath till crystal dissolved.

Materials Required but Not Supplied

- ✓ 20 µL and 100 µL micropipettors and disposable tips.
- ✓ Incubator or water bath with temperature control at 37°C
- ✓ Automatic microplate washing equipment.
- ✓ ELISA microwell reader: Dual wavelength 450/650 nm.

Precautions for Use

- ✓ For professional use only.
- ✓ For research use only.
- ✓ Bring all kit reagents and samples to room temperature (20 to 30°C) and mix gently before use.
- ✓ Do not use reagent beyond its expiration date.
- ✓ Do not interchange reagents between different lots.
- ✓ Avoid microorganism contamination.
- ✓ Do not pipette in the mouth.
- ✓ Do not smoke or eat in areas where specimens or reagents are handled.
- ✓ The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
- ✓ Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
- ✓ Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:
Both liquid and solid waste should be autoclaved maintaining 121°C for at least 1 hour.
Solid waste can also be incinerated.
Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%

acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.

- ✓ 2 N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2 N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
- ✓ TMB substrate solution A contains an organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes. In case of contact, clean with large lots of water.

Assay Protocol

Reagent Preparation

- ✓ Preparation of Diluted Conjugate
 - Bring Conc. HIV Antigen Conjugate and Conjugate Diluent D to room temperature (20-30°C) before use.
 - Use only clean container to avoid contamination.
 - Prepare diluted conjugate by making 1:101 with Conjugate Diluent D, or following Conjugate Preparation Chart below. Swirl gently to mix thoroughly and avoid foaming.
 - Excess diluted conjugate solution should be discarded after use.

Conjugate Preparation Chart:

Number of Wells used	Volume of Conjugate Diluent D needed (mL)	Volume of Conc. HIV Antigen Conjugate needed (µL)
8	1	10
16	2	20
24	3	30
32	4	40
40	5	50
48	6	60
56	7	70
64	8	80
72 – 80	9	90
81 - 96	10	100

- ✓ Plate Washing Procedure

Dilute Washing Solution D (20X) Concentrate with distilled or deionized water to 1: 20 dilution. Do not use tap water.

Automatic Microplate Washing Procedure:

- Any commercial automatic microplate washer or other liquid aspirating/dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. We suggest that 6 cycles with at least 0.4 mL wash buffer per well per wash and soaking for 10 seconds.
- Blot dry by inverting the plate and tapping firmly onto absorbent paper. All residual washing buffer should be blotted dry.

Note: Improper washing will cause false results.

Sample Preparation

- ✓ Either serum or plasma can be used with this kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Also, clots must be removed.
- ✓ Specimens must be stored at 2-8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
- ✓ Frozen specimens must be thawed thoroughly and mixed before test.
- ✓ Avoid multiple freeze-thaw procedures.

Assay Procedure

Assay process can be performed manually or by automatic EIA microplate immunoanalyzer.

1. Bring all Reagents and Specimens to room temperature (20-30°C) before begin the assay. Adjust water bath or incubator to 37±1°C.
2. Prepare the needed number of wells, including two wells for Blanks, two wells for Negative Control, two wells for Anti-HIV1 Positive and two wells for Anti-HIV2 Positive Control, and one well for each Specimen.
3. Add 100 µL of Negative Control, HIV-1 Positive and HIV-2 Positive Control to each appropriate well. (except blank wells).

Note: Use a new pipette tip for each sample to avoid cross contamination.

4. Seal the Plate with an Adhesive Slip to avoid vapor.
5. Incubate the plate in a 37±1°C water bath or humidified incubator for 30 minutes.
6. At the end of the incubation period, remove and discard the Adhesive Slip and wash the Plate for 6 times (see Plate Washing Procedure).
7. Prepare diluted conjugate as the Conjugate Preparation Chart
8. Add 100 µL of the diluted Conc. HIV Antigen Conjugate in each well.
9. Seal the plate with an Adhesive Slip
10. Incubate the plate in a 37±1°C water bath or humidified incubator for 20 minutes.

Note: Do not stack plates.

11. At the end of the incubation period, remove and discard the Adhesive Slip and wash the Plate for 6 times (see Plate Washing Procedure).
12. Choose one of the following two methods for color development:

- ✓ Mix an equal volume of TMB Substrate Solution A and B in a clean container before use. Add 100 µL of the TMB Substrate Solution A and B mixture to each well including 2 blanks.

Note 1: TMB Substrate Solution A should be colorless, otherwise, it should be discarded.

Note 2: The mixture of TMB Substrate Solution A and B must not be stored longer than 10 minutes after preparation. Do not expose the mixture of TMB solution under intense light. The mixture should be colorless or light blue, otherwise, it should be discarded.

- ✓ Add 50 μL of TMB Substrate Solution A and 50 μL of TMB Substrate Solution B into each well including 2 blanks. Mix well gently.
- 13. Cover the plate with Black Cover and incubate the plate at room temperature (20-30°C) for 15 minutes.
- 14. Stop the reaction by adding 100 μL of 2 N Sulfuric Acid to each well, including blank wells.
- 15. Measure the optical density within 15 minutes by a precision ELISA reader. Blank the instrument by using the lighter one of the two blanks. Read the absorbance at wavelength of 450 nm or 450/650 nm.

Note 1: The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid.

Note 2: Blank well: absorbance value must be less than 0.100.

Data Analysis

Calculation of Results

- ✓ Calculation of NCx:

Example: NC Absorbance

1 0.022

2 0.024

$$\text{NCx} = (0.022 + 0.024) / 2 = 0.023$$

- ✓ Calculation of Anti-HIV1 PCx and Anti-HIV2 PCx:

Example:

Anti-HIV1 PC Absorbance

1 1.045

2 1.170

$$\text{Anti-HIV1 PCx} = (1.045 + 1.170) / 2 = 1.108$$

Anti-HIV2 PC Absorbance

1 1.116

2 1.097

$$\text{Anti-HIV-2 PCx} = (1.116 + 1.097) / 2 = 1.107$$

Both Anti-HIV1 PCx and Anti-HIV2 PCx should be ≥ 0.50 , otherwise, the test is invalid.

- ✓ Determination of Cut-off Value:

$$\text{Cut-off Value} = \text{NCx} + 0.10$$

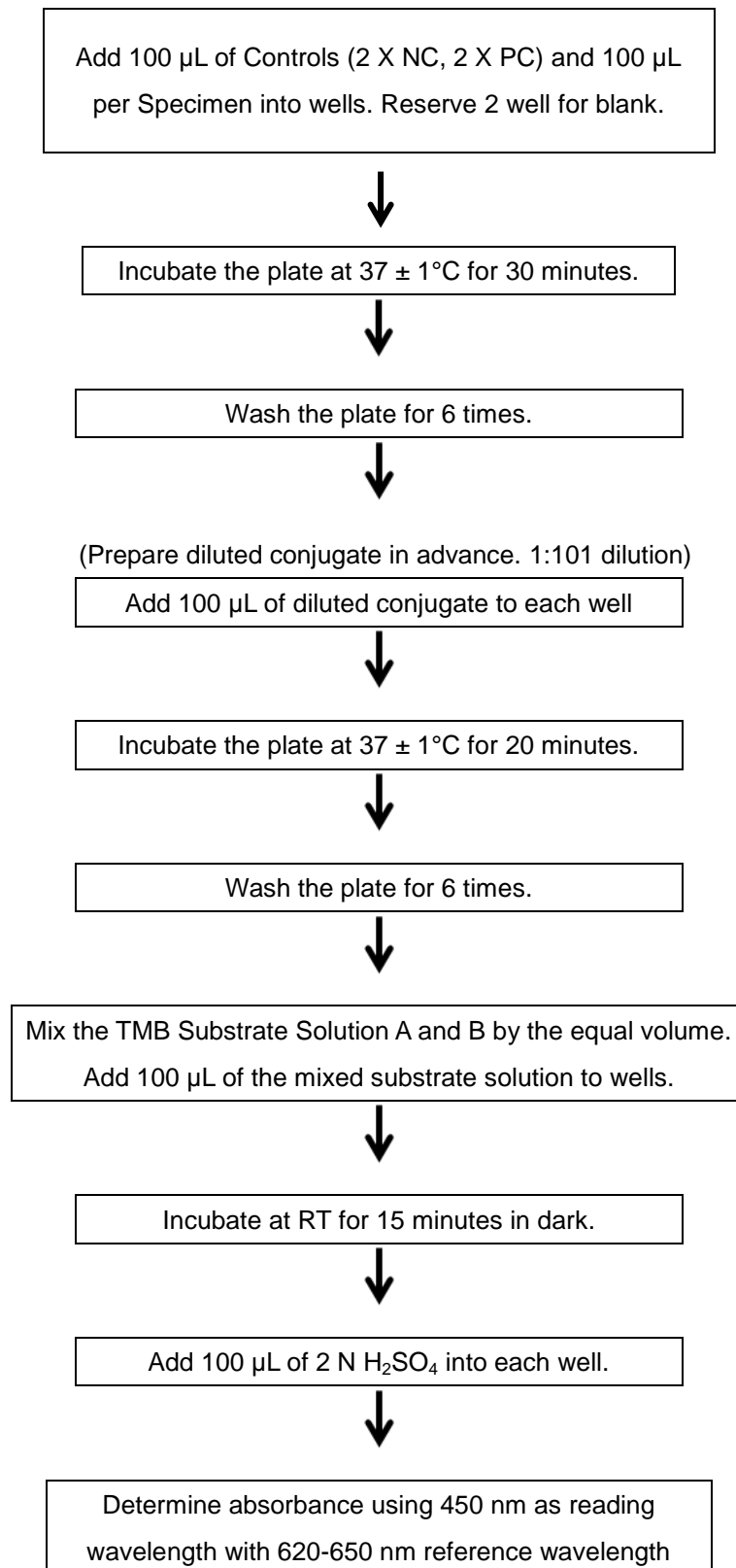
Example:

$$\text{Cutoff Value} = 0.023 + 0.10 = 0.123$$

Note:

- ✓ *Non-reactive Result: Specimens with absorbance values LESS than the CUTOFF VALUE are considered NON-REACTIVE by the criteria of HIV-1/2 Ab ELISA Kit.*
- ✓ *Reactive Result: Specimens with absorbance values EQUAL to or GREATER than the CUTOFF VALUE are considered INITIALLY REACTIVE and should be RETESTED in duplicate using the original sample source.*
- ✓ *If duplicate retests are non-reactive, the specimen is considered NON-REACTIVE by the criteria of HIV-1/2 Ab ELISA Kit. If either duplicate retest is reactive, the specimen is considered REPEATEDLY REACTIVE.*
- ✓ *Specimens found repeatedly reactive by HIV-1/2 Ab ELISA Kit must be further tested by other confirmatory tests (such as Western Blot). If specimens tested reactive with these tests, the specimens are considered POSITIVE or presence of antibody to HIV-1 and/or HIV-2.*

✓ Flow chart of the test procedure



Resources

References

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5. T. Mathiesen, 1989. Mapping of IgG subclass and T-cell epitopes on HIV proteins by synthetic peptides, *Immunology*, 67, 453-459.
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Plate Layout

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4								
3								
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1								
	A	B	C	D	E	F	G	H