RecombiLISA

HIV 1+2 Ab ELISA

IVD **REF** **E0410**

- 96-well ELISA Test for the qualitative detection of anti-HIV-1 including subtype O and anti-HIV-2 antibodies in human serum or plasma
- For export only, not for re-sale in the USA
- Store at 2-8°C upon receipt

INTENDED USE

The *RecombiLISA* HIV 1+2 Ab ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of anti-HIV-1 including subtype O and anti-HIV-2 antibodies (including isotype IgG, IgM and IgA) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HIV-1 and HIV-2 viruses.

INTRODUCTION

Human immunodeficiency virus (HIV) is an enveloped, single stranded, positive sense RNA virus that attacks the body's immune system, specifically cells that help the immune system fight off infection, CD4 cells (T cells). It reduces the number of T cells, putting the person at higher risk for infection[1]. If left untreated, HIV can lead to acquired immunodeficiency syndrome (AIDS).

Two types of HIV have been characterized, HIV-1 and HIV-2. HIV-1 has been isolated globally from patients with AIDS and AIDS-related complex, and from healthy individuals with a high risk for developing AIDS[2]. It has been divided into four groups (M, N, O, and P). Group M is the cause of the last century's global HIV pandemic, and consists of nine subtypes: A–D, F–H, J, and K. HIV-2 is largely confined to west Africa and causes a similar illness to HIV-1, but is slower progressing and less transmissible[1,3].

Infection with HIV induces the immune system to produce antibodies against viral proteins from different parts of the HIV genome, ENV, GAG and POL. In most cases, HIV antibodies will become detectable 3 to 12 weeks (21 to 84 days) after infection[4]. Diagnosis of anti-HIV seropositivity is based on the detection of these specific antibodies.

The *RecombiLISA* HIV 1+2 Ab ELISA is a third generation HIV test for the qualitative detection of anti-HIV-1 and HIV-2 antibodies.

TEST PRINCIPLE

RecombiLISA HIV 1+2 Ab ELISA is a solid-phase enzyme-linked immunosorbent assay based on the principle of the double antigen-sandwich technique for the detection of the various antibodies against HIV-1 and/or HIV-2 in human serum or plasma.

The *RecombiLISA* HIV 1+2 Ab ELISA is composed of two key components:
1) Solid microwells pre-coated with recombinant HIV-1 and HIV-2 antigens;
2) Liquid conjugates composed of recombinant HIV-1 and HIV-2 antigens conjugated with horseradish peroxidase (HRP-HIV-1+2 Conjugate).

During the assay, the test specimen is first incubated in the coated microwells. The anti-HIV-1 and anti-HIV-2 antibodies, if present in the specimen, bind to the antigens coated on the microwell surface, and any unbound specimen is then removed by a wash step.

During a second incubation with the HRP- HIV-1+2 Conjugate, the antibodies bound to the microwell surface bind to the HIV-1+2 antigens in the HRP conjugate, forming a conjugate complex. Unbound conjugates are then removed by washing. After addition of the TMB Substrate, the presence of the conjugate complex is shown by a blue color resulting from a reaction between the enzyme and substrate. The reaction is then quenched by addition of the Stop Solution, and the absorbance value for each microwell is determined using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 50 µL and 100 µL
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. Distilled or de-ionized water

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	HIV-1 & HIV-2 Ag Coated Microwells	8 wells x 12 strips	E0410W
2	HIV-1 Ab Positive Control	1 mL	E0410P1
3	HIV-2 Ab Positive Control	1 mL	E0410P2
4	HIV Ab Negative Control	1 mL	E0410N
5	HRP-HIV-1+2 Conjugate	6 mL	E0410H
6	Wash Buffer (30 x Concentrate)	20 mL	WE3000
7	TMB Substrate A	6 mL	TME2000A
8	TMB Substrate B	6 mL	TME2000B
9	Stop Solution	6 mL	SE1000
10	ELISA Working Sheet	2	E0001ES
11	Product Insert	1	PI-E0410
Others	3 x Microplate Sealers and 1 x Resealable Bag		

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Reseal the microwells after removing the desired number of wells. Place unused wells in the resealable bag provided and return to 2-8°C. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (18-28˚C).
2. **Preparation of working Wash Buffer** Warm up the concentrated Wash Buffer to 37°C to dissolve the precipitant if it appears. Dilute concentrated Wash Buffer 30 fold with water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2.0 mL	60 mL
2 strips	116 mL	4.0 mL	120 mL
3 strips	174 mL	6.0 mL	180 mL
4 strips	232 mL	8.0 mL	240 mL

The diluted wash buffer can be stored at 2-8°C for up to 3 days.

3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA Working sheet with the appropriate information. Positive and Negative Controls should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable plastic bag, seal and store at 2-8°C for later use.
2. Add specimens according to the designation on the ELISA Working Sheet:

2.1 Blank Well: Do not add any reagents.

2.2 Control Wells: Add 50 µL of HIV-1 Ab Positive, HIV-2 Ab Positive, and Negative Control into the designated control wells, respectively.

2.3 Test Wells: Add 50 µL of test specimen to each test well.

3. Cover the plate with a sealer. Incubate the wells at 37°C for 30 minutes.
4. Wash Step (Can be performed manually or with automated washing):

Manual washing: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL of diluted wash buffer and shake gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.

Automatic washing: Automatic plate washer must be calibrated to ensure efficient washing. Aspirate incubation mixture from all wells completely. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.

5. Add 50 µL of HRP-HIV-1+2 Conjugate into each well, except the Blank Well. Cover the plate with a sealer.
6. Incubate at 37°C for 30 minutes.
7. Wash the plate 5 times as described in step 4.
8. Add 50 µL of TMB Substrate A and 50 µL of TMB Substrate B into each well including the Blank Well.
9. Incubate at 37°C in dark for 15 minutes.
10. Stop the reaction by adding 50 µL of Stop Solution into each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color completely changes to a color yellow.**
11. Set the microplate reader wavelength at 450 nm. Measure the absorbance (OD) of each well against the Blank Well within 15 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add HIV-1 Ab Positive, HIV-2 Ab Positive, Negative Control, and specimens, respectively	50 µL
3.	Incubate	37°C, 30 minutes
4.	Wash: manual or automatic	5 times
5.	Add HRP-HIV-1+2 Conjugate, except Blank Well	50 µL
6.	Incubate	37°C, 30 minutes
7.	Wash: manual or automatic	5 times
8.	Add TMB Substrate A and B, respectively	50 µL + 50 µL
9.	Incubate in dark	37°C, 15 minutes
10.	Add Stop Solution. Gently mix	50 µL, 30 seconds
11.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.08 + N

N: Mean OD of the negative control. Use N=0.05 for calculation of the cut-off value if the mean OD is less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the HIV Ab positive controls should be ≥ 1.5.
The mean OD value of the HIV Ab negative controls should be ≤ 0.10.

Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1. A negative result indicates that there are no detectable HIV antibodies in the specimen.
2. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the *RecombiLISA* HIV 1+2 Ab ELISA. They should be retested in duplicate before a final interpretation is made.
3. Results within 10% of the cut-off value should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
   If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the *RecombiLISA* HIV 1+2 Ab ELISA, subject to the limitations of the procedure, described below.
   If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the *RecombiLISA* HIV 1+2 Ab ELISA.
   Non-repeatable reactions are often caused by:
   - Inadequate microwell washing
   - Contamination of negative specimens by serum or plasma with a high antibody titer
   - Contamination of the TMB Substrate by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop Solution

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

53 HIV-1 and 5 HIV-2 confirmed positive clinical specimens and 1037 normal clinical specimens were tested with *RecombiLISA* HIV 1+2 Ab ELISA in three different lots and compare the results with and by a Chinese State Drug Administration (CFDA) licensed reference EIA. Comparison for all subjects is shown in the following table:

	***RecombiLISA* HIV 1+2 Ab ELISA**		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	58	0	58
Negative	1	1036	1037
Total	59	1036	1095

Relative Sensitivity: 100% (95% CI: 92.0% - 100%)
Relative Specificity: 99.9% (95% CI: 99.9% - 100%)
Overall Agreement: 99.9% (95% CI: 99.9% - 100%)

2. Cross-reactivity

No false positive *RecombiLISA* HIV 1+2 Ab ELISA results were observed on positive specimens from each of the following disease states or special conditions, respectively:

HBsAg HCV Syphilis Dengue Malaria Typhoid

3. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the *RecombiLISA* HIV 1+2 Ab ELISA. Interference was studied by spiking these substances into 3 levels of HIV 1+2 Ab clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *RecombiLISA* HIV 1+2 Ab ELISA.

List of potentially inferring substances and concentrations tested:

Potential Interfering Substance	Tested Concentration
Bilirubin	20 mg/dL
Glucose	55 mM
Salicylic Acid	4.34 mM
Sodium Citrate	3.8 %
EDTA	3.4 µM
Creatinine	442 µM
Heparin	3000 U/L

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (18-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. At the beginning of each incubation, and after adding Stopping Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme-substrate reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or TMB Substrate.
13. The TMB Substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate B must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
15. **The wash procedure is critical. Wells must be aspirated completely before adding the Washing Buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.**
16. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated reader often leads to invalid test results.**
17. Avoid exposure to strong light during color development.

LIMITATION OF THE TEST

1. The Assay Procedure and the Interpretation of Result must be followed closely when testing for the presence of anti-HIV antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HIV 1+2 Ab ELISA is limited to the qualitative detection of HIV antibodies in human serum or plasma. The intensity of color does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable anti-HIV-1 and HIV-2 antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with HIV-1 and HIV-2.
4. A negative result can occur if the quantity of anti-HIV-1 and HIV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect results.
6. Any use or interpretation of this test's results must also rely on other clinical findings and the professional judgment of health care providers.

REFERENCES

1. Maartens G, Celum C, Lewim SR, Gary. HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet. 2014, 384(9939);258-71
2. Chang, SY, Bowman, BH, Weiss, JB, Garcia, RE and White, TJ. The origin of HIV-1 isolate HTLV-IIIB. Nature (1993)3/363:466-9
3. Arya, SK, Beaver, B, Jagodzinski, L, Ensoli, B, Kanki, PJ, Albert, J, Fenyo, EM, Biberfeld, G, Zagury, JF and Laure, F. New human and simian HIV-related retroviruses possess functional transactivator (tat) gene. Nature (1987) 328:548-550
4. Centers for Disease Control and Prevention. (2016). Act Against AIDS. http://www.cdc.gov/nutrition/https://www.cdc.gov/actagainstaids/basics/testing.html

Index of Symbols

	See instructions for use		Use by
IVD	For *in vitro* diagnostic use only	CONJ	Conjugates
REF	Catalog #	MICROWELLS	Coated microwells
LOT	Lot number	CONTROL +	Positive control
Σ N	Tests per kit	CONTROL -	Negative control
2	Do not reuse	TMB SUBS	TMB substrate
	Manufacturer	WASH BUFF	Wash buffer
	Date of manufacture	STOP SOLN	Stop solution
2°C 8°C	Store between 2-8°C		

CTK Biotech, Inc.
13855 Stowe Drive,
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

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