

# HIV 1/2 ELISA Kit

The third generation double antigen sandwich method to detect antibody to HIV1+2 in serum or plasma

## INTENDED USE

This EIA kit is **the third generation double antigen sandwich method** for the detection of circulating antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and/or Human Immunodeficiency Virus Type 2 (HIV-2) in Human serum or plasma and is indicated as a screening test for serum or plasma and as an aid in the diagnosis of potential infection with HIV-1 and/or HIV-2.

## SUMMARY AND PRINCIPLE OF THE TEST

Human Immunodeficiency Virus Type (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC). HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of Human Immunodeficiency Virus (HIV-2) was isolated and also reported to cause AIDS. Since the initial discovery, more than 600 cases of HIV-2 infection have been documented worldwide, with over 40 cases of AIDS related to HIV-2. Both viruses have the same morphology and lymphotropism, and the modes of transmission appear to be identical. In addition, HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as *gag* and *pol*. Serologic studies have also shown that the core proteins of HIV-1 and HIV-2 display frequent cross-reactivity whereas the envelope proteins are more type-specific.

Despite this immunologic cross-reactivity, detection of antibodies to HIV-2 with any of the licensed HIV-1 enzyme immunoassays is highly variable. This HIV-1/HIV-2 EIA was developed to detect antibodies to HIV-1 and/or HIV-2, for blood screening and diagnostic purposes.

Any specimen that reacts in an initial test with the HIV-1/HIV-2 EIA must be retested in duplicate with the other company's HIV-1/HIV-2 EIA. Repeatedly reactive specimens may contain antibodies to either HIV-1 or HIV-2. Therefore, additional, more specific or supplemental tests for antibodies to both HIV-1 and HIV-2 such as immunoblot, immunofluorescence, radioimmuno-precipitation must be performed to verify presence of antibodies to HIV.

## Principles of the Assay

The HIV-1/HIV-2 EIA utilizes a detection system where microplate wells are coated with synthetic peptides and recombinant antigen corresponding to a highly antigenic segment of HIV-1/HIV-2 envelope and core proteins. Serum or plasma specimens, controls are added to the wells. During incubation, antibodies specific for HIV-1 and HIV-2 present in the specimen will bind to the peptides and recombinant antigen fixed onto the microplate wells. The wells are washed to remove unbound materials, and recombinant antigen conjugate will bind to the antigen-antibody complex and excess unbound enzyme conjugates are again removed by washing. The enzyme substrate, tetramethylbenzidine (TMB), is added upon incubation the substrate will be hydrolyzed by the bound enzyme and a blue or blue-green colour develops in wells containing HIV-1 and/or HIV-2 specific antibodies. The enzyme reaction is stopped by the addition of sulphuric acid. The intensity of colour developed is read spectrophotometrically at 450nm and is proportional to the amount of antibodies present in the specimen.

## REAGENTS

### Materials provided with the kits:

- 12X8 well microtiter strip: 1 plate, coated with HIV-1/HIV-2 specific synthetic peptides and recombinant antigen.
- Negative Control: One vial Normal human serum non-reactive for HBsAg and antibodies to HCV, HIV-1 and HIV-2. Contains sodium azide as preservative.
- Positive Control: One vial Inactivated human serum with high titer antibodies to HIV-1 and non-reactive for HBsAg and for HCV. Contains sodium azide as preservative.
- Enzyme Conjugate: Phosphate buffered saline with Tween-20 containing normal goat serum, protein stabilizer and recombinant gp41B, gp36 and gp120 antigen peroxidase (horseradish) conjugate. Uses Proclin 300 as preservative.
- Specimen Diluent: 12 ml PBS contain tween-20 and BSA.
- Wash Buffer Concentrate (20x): 40 ml, containing PBS, Tween. The buffer should be diluted 20 times with distilled water before use.
- Substrate Solution A: urea peroxide.
- Substrate Solution B: TMB.
- Stop Solution: 2N Sulfuric Acid

### Materials required but not provided:

- Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
- Disposable pipette tips.
- Distilled or deionized water.
- Humidified Box capable of maintaining 37°C
- Absorbent paper or paper towel.
- Microtiter plate or strip-well washer
- Microtiter plate reader with 450nm wavelength
- Timer

## PRECAUTION FOR USERS

- For in-vitro diagnostic use only.
- Do not use kit beyond expiration date.
- Do not mix components from kits with different lot number.
- Avoid microbial contamination of reagents.
- Do not pipet reagent by mouth and no smoking or eating while performing assays.
- Wear gloves during the whole process and avoid reagents or specimen spilling-out.
- Wipe up the spills using 5% hypochlorite solution.
- Decontaminate all liquids or solid wastes before depositing.

## SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

## ASSAY PROCEDURE

- Allow all components to reach room temperature before use.
- Dispense 100 ul of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, for test samples add 50ul Specimen Diluent and 50ul of serum or plasma samples into respective wells.
- Place the microtiter plate into a humidified box and incubate at 37°C for 40 min.
- Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
- Add 100 ul of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
- Place the microtiter plate into a humidified box and incubate at 37°C for 40 min.
- Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
- Add 50 ul of Substrate Solution A (HRP substrate) to each well, then add 50 ul of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 20 min.
- Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

## INTERPRETATION OF RESULTS

**EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):**

- A run is valid if:
  - The full complement of Blanks, Positive and Negative Controls must be included in each assay.
  - Negative Control values must have an absorbance and  $\leq 0.10$  after subtracting the Blank.
  - Anti-HIV-1 Positive Control value must have absorbance  $\geq 0.600$  after subtracting the Blank.

2. Calculation of Cut Off Value(COV)

Mean of the Negative Controls (NCx)+0.10

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

**Interpretation of the Results**

1. Specimens with absorbance values less than the cutoff value (i.e. OD/COV<1.00) are considered to be negative.
2. Specimens with initial absorbances greater than or equal to the cutoff value (i.e. OD/COV≥1.00) are considered initially positive by the criteria of this EIA and should be retested in duplicate before interpretation.
3. Specimens found positive on retesting may be interpreted to be repeatedly positive for antibodies to HIV-1 and/or HIV-2 by the criteria of this EIA.
4. Initially reactive specimens which are negative in both wells on the repeat test are considered negative for antibodies to HIV-1 and HIV-2.
5. Specimens which are repeatedly positive in this EIA should be further tested by additional, more specific tests.

**LIMITATIONS OF THE ASSAY**

Repeatedly reactive results in the HIV-1/HIV-2 EIA are presumptive evidence of antibodies in the specimen. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1/HIV-2 is present.











The primary use of the HIV-1 and HIV-2 EIA is to screen blood and plasma donations so that units containing antibody can be identified and eliminated, or restricted to further manufacturing into non-injectable products.

A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1/HIV-2.

**Manufacturer:**

**Biocare Diagnostics Ltd.**  
6F, Building B, 108 Xinghua Road  
Xiangzhou, Zhuhai, China 519000  
Tel: +86-756-8238560  
E-mail: [info@ivdbiocare.com](mailto:info@ivdbiocare.com)  
Website: [www.ivdbiocare.com](http://www.ivdbiocare.com)

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	<i>In vitro</i> diagnostic device		Lot code
	Consult instructions for use		Catalogue number
	Keep dry		Contains sufficient for <n> tests
	Temperature limitation		Manufacturer
	Use by		Do not use if package damaged