



## HIV 1&2

Enzyme Immunoassay for the qualitative determination of antibodies to Human Immunodeficiency Virus Type 1 and Type 2 in human plasma and sera. For professional *in vitro* diagnostic use only - 96 tests.

### Catalogue No. 7/003

#### INTRODUCTION

Human Immunodeficiency Virus Type 1 (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&2 kit 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 assay must be retested in duplicate. 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 the presence of antibodies to HIV.

#### Intended Use

This kit is intended for use 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.

#### Principles of the Method

The Biotec HIV 1&2 assay utilises 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 and 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 the recombinant antigens conjugated with horseradish peroxidase are added. The enzyme conjugates will bind to the antigen-antibody complex and excess unbound enzyme conjugates are again removed by washing.

The enzyme substrate, tetramethylbenzidine (TMB), is hydrolysed during incubation 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.

#### PRODUCT CONTENTS

##### For 96 test assay

- Coated Microplate:** 12 x 8 well strips per plate.  
Each microplate well contains fixed HIV-1/HIV-2 specific synthetic peptides and recombinant antigen.
- Negative Control:** 1 vial of 1ml.  
Normal human serum non-reactive for HBsAg and antibodies to HCV, HIV-1 and HIV-2. Contains sodium azide as preservative.
- Positive Control:** 1 vial of 1ml.  
Inactivated human serum with high titre antibodies to HIV-1 and non-reactive for HBsAg and for HCV. Contains sodium azide as preservative.
- Conjugate:** 1 vial of 12ml.  
Phosphate buffered saline with Tween-20 containing normal goat serum, protein stabiliser and recombinant gp120, gp41, gp36 antigen peroxidase (horseradish) conjugate. Contains thimerosal as preservative.
- Wash Solution** 1 bottle of 80ml.  
25x Concentrate.
- Substrate Buffer:** 1 bottle of 8ml.  
Contains hydrogen peroxide.
- TMB:** 1 bottle of 8ml.  
Citric buffer containing tetramethylbenzidine (TMB) and dimethylsulfoxide (DMSO).
- Stopping Solution:** 1 bottle of 7ml  
2M sulphuric acid solution. **Warning** Corrosive, refer also to 'Warnings & Precautions' no. 23.
- Plate Covers:** 4 pieces.  
Plastic covers for microplate during incubation.
- Plastic Pouch and Desiccant:** For unused strips.
- Instructions for use:** 1 copy

**Note:** Store the kit at 2-8°C. Bring all reagents except Conjugate to room temperature before use. Remove the Conjugate from 2-8°C immediately before use. Return to 2-8°C storage immediately after use.

#### ITEMS REQUIRED BUT NOT PROVIDED

- Distilled water.
- Manual or automatic pipettes capable of delivering 50µl, 100µl and 1000µl.
- Disposable pipette tips.
- Timer.
- Microplate mixer.
- Incubator (37°C).
- An automatic microplate washer (strongly recommended).
- Microplate reader (equipped with a 450nm and 620nm filter).
- Gloves.

#### STORAGE AND SHELF LIFE

- If kept at 2-8°C, all the test reagents are stable until the expiry date printed on the box label.
- When the aluminium bag has been opened, the unused strips can be safely stored at 2-8°C in the sealed plastic pouch along with the silica gel placed inside.
- After opening, the conjugate, TMB, substrate, concentrated washing solution and controls are stable until the expiry date, if kept at 2-8°C sealed in the original vials.



## WARNINGS & PRECAUTIONS

1. Upon receipt, store the kit at 2-8°C.
2. All the reagents are for professional *in vitro* diagnostic use only.
3. Do not use the kit or reagents beyond the expiry date specified on the labels or if damaged. Do not mix reagents from different lots.
4. Procedures should be performed carefully in order to obtain reliable results and interpretation.
5. Allow all reagents to reach room temperature at least 60 minutes before use, except Conjugate.
6. Use only reagent grade quality, deionised or distilled water to dilute reagents where instructed.
7. When using the kit, check that the reagent solutions are clear. Any cloudiness or odour is indicative of contamination.
8. Avoid cross-contamination between serum specimens.
9. Avoid cross-contamination of reagents when removing them from the vials. Automatic pipettes with disposable tips are recommended. To avoid cross-contamination when dispensing reagents, do not touch the side of the wells with tips.
10. Avoid cross-contamination between conjugate and substrate.
11. The use of disposable glass or plastic-ware is recommended in order to avoid contamination.
12. For the washing step, use only the Washing Solution provided in the kit. Carefully follow the instructions. The use of a good quality microplate washer is recommended.
13. Avoid contact between the substrate/chromogen and oxidising agents or metallic surfaces. Avoid intense light exposure during incubation or reagent preparation. The use of plastic disposable (sterile) containers is recommended for the preparation of chromogen/substrate.
14. Samples and materials potentially infected must be handled with care. All objects which are in direct contact with samples and all residuals of the assay should be treated as potentially infected.
15. There is no test method available that can offer complete assurance that infectious agents are absent. Therefore all human serum specimens and kit reagents should be handled at Biosafety Level 2 as recommended by the Centers for Disease Control/U.S. Institutes of Health publication "Biosafety in Microbiological and Biomedical Laboratories", 1984.
16. Wipe any spills promptly with 1% sodium hypochlorite solution.
17. The stopping solution is strong acid. Wipe spills immediately. Flush the area of the spill with water. If the stopping solution contacts the skin or eyes, flush with copious amounts of water and seek medical attention.
18. To inactivate wash solutions and reagents utilised in the assay, Autoclave all used and contaminated materials at 121°C, 15psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal. Other materials should be treated as biohazardous waste and disposed of according to recommended procedures.
19. Avoid contact with skin and mucous membranes, especially with the stopping reagent.
20. Avoid repeatedly opening and closing the incubator during incubation steps.
21. Use protective talc-free gloves, safety spectacles and laboratory coat when working with samples. Never pipette by mouth. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
22. All the instruments (washer, reader etc) must be well maintained and serviced regularly.
23. The stopping solution contains 2M sulphuric acid. This reagent is corrosive and carries the following risk and safety phrases: **R35** causes severe burns. **S26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **S30** never add water to this product. **S45** in case of accident or if you feel unwell seek medical advice immediately (show the label where possible).
24. Always follow standard laboratory safety procedures for biohazard containment and report any incident or accident according to local Health and Safety Guidelines.

## PREPARATION OF REAGENTS

### 1. WORKING SUBSTRATE SOLUTION:

The working substrate solution is a 1:1 combination of the substrate with the TMB reagent. For every two strips to be tested, add 1ml of the TMB to 1ml of the substrate as shown in the following table.

#### Note:

- a) Do not mix the entire Substrate Buffer with the TMB solution; extra reagents are provided.
- b) It is recommended that the working substrate solution should be used within 20 minutes of preparation.
- c) The working substrate solution should be colourless. A distinct blue colour indicates that the solution is contaminated. Discard the working substrate solution and prepare fresh solution in a clean container.

Number of strips to be used	2	4	6	8	10	12
Amount of HIV-1/HIV-2 Substrate Buffer (ml)	1	2	3	4	5	6
Amount of HIV-1/HIV-2 TMB Solution (ml)	1	2	3	4	5	6

### 2. WASH SOLUTION

The final working wash solution is a 1:25 dilution of the Wash Solution Concentrate (25X) provided with the kit. Prepare wash solution as needed by adding one part concentrate (25X) to twenty-four parts deionised water. The diluted wash solution can be stored at room temperature for up to 1 week.

**Note:** Crystals may form when Wash Solution Concentrate is stored at 2-8°C. This must be dissolved by warming to 37°C prior to use.

## PROCEDURE

- Bring all reagents except the Conjugate to room temperature before beginning the assay procedure.
- Remove microplate from the aluminium bag, put unused strips and desiccant into the plastic pouch and reseal the pouch.
- Shake specimen and control vials before use.
- Add 100µl of Negative Control to each of two wells, use a clean pipette tip for addition.
- Add 100µl of Positive Control to each of three wells, use a clean pipette tip for addition.
- Using pipette, introduce 100µl of specimen to the assigned wells. **NOTE:** In every test leave two wells as blank and do not add specimen or conjugate to these two wells. Use a clean pipette tip for each specimen.
- Cover the microplate with the plate cover to minimise evaporation and incubate the plate for 60 minutes at 37°C.
- Remove the Conjugate from 2-8°C storage immediately prior to use.
- Carefully remove the plate cover and aspirate the fluid from each well into a biohazard container. Prior to disposal, make sure enough disinfectant is added to the container.
- Wash the microplate five times with diluted wash solution. Aspirate the wash solution each time, after the last wash, blot the inverted plate on absorbent paper towels.
- Add 100µl of working conjugate solution to each well containing specimen or control.
- Cover the plate with a fresh plate sealer and incubate the plate at 37°C for 30 minutes.
- Prepare working substrate solution as described in 'Preparation of Reagents'.
- Remove and discard the plate cover. Repeat the wash procedure as in step 10.
- Add 100µl of the working substrate solution per well. Cover the plate with a fresh sealer and incubate the plate for 10 minutes at 37°C.
- Carefully remove the plate sealer and add 50µl of stopping solution to each well to terminate the reaction.
- Read the absorbance for each well at 450nm. If a dual filter instrument is used, the reference wavelength should be 620nm.

### Note:

- Once the assay has been started, it should be completed without interruption.
- Absorbance should be read within 1 hour of the addition of the Stop Solution.
- Do not use the microwell washer to aspirate acid and do not aspirate acid into bleach.

## Assay Schematic

HIV 1&2 antigen coated wells			
REAGENTS	BLANK (2 wells)	CONTROLS*	SPECIMEN
<b>Controls</b>	-	100µl	-
<b>Specimen</b>	-	-	100µl
Cover strips with plate cover			
<b>Incubate for 60 min. at +37°C.</b>			
Remove the plate cover and aspirate the reaction solution from all wells into a biohazard container.			
Wash 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid. After the last wash, blot the inverted plate on absorbent paper towels.			
Remove the Conjugate from 2-8°C storage immediately prior to use			
<b>Diluted Conjugate</b>	-	<b>100 µl</b>	<b>100 µl</b>
Cover strips with new plate sealer			
<b>Incubate for 30 min. at +37°C.</b>			
Remove and discard the plate cover and aspirate the reaction solution from all wells. Repeat washing step as above.			
Prepare working substrate solution as described in 'Preparation of Reagents'			
<b>TMB/Substrate (Mixed 1:1)</b>	<b>100 µl</b>	<b>100 µl</b>	<b>100 µl</b>
Cover strips with a new plate sealer.			
<b>Incubate for 10 min. at 37°C</b>			
Remove the plate sealer			
<b>Stopping solution</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>
Read the absorbance of each well at 450 nm and 620 nm (as a reference), within 1 hour of adding the stopping solution.			

\*Refer also to 'Procedure' no. 4 and 5.

## INTERPRETATION

### Results

The presence or absence of antibodies to HIV-1 and/or HIV-2 is determined by relating the absorbance value of the specimen to the cut-off value.

### Quality Control

- A run is valid if all the following requirements are met:
  - The full complement of blanks, positive and negative controls must be included in each assay.
  - Blank values must have an absorbance <0.100.
  - Negative control values must have an absorbance <0.080 after subtracting the blank.
  - Anti-HIV-1 positive control value must have absorbance >0.800 after subtracting the blank.
- Calculation of Control:

Determine the mean of the positive control absorbance (PCx) as shown in the example below:

Positive Control			
Repeat No.	1	2	3
Absorbance	1.125	1.330	1.227
<b>PCx = 1.227</b>			



### 3. Calculation of the Cut-Off:

The cut-off value is 10% of the mean of positive controls. Calculate the cut-off value as shown in example below:

$$PCx = 1.227$$

$$\text{Cut-off} = 1.227 \times 10\% = 0.123$$

### 4. Calculation of the Specimen:

Calculate the absorbance for each specimen by subtracting the value of the blank from the value of each specimen. If the microplate reader performs the blank subtracting process, leave out this step. Determine the test result of the specimen as shown in the example below:

Specimen	Absorbance	OD/Cut Off	
1	0.069	0.069/0.123=0.561	<1.00
2	0.482	0.482/0.123=3.919	>1.00

In the above examples, Specimen No.1 (0.069) is negative and Specimen No.2 (0.482) is positive for antibodies to HIV-1and/or HIV-2 when compared to the cut-off value of 0.123.

### Interpretation of the Results

- Specimens with absorbance values less than the cut-off value (i.e. OD/CO <1.00) are considered to be negative.
- Specimens with initial absorbance greater than or equal to the cut-off value (i.e. OD/CO >1.00) are considered initially positive by the criteria of this assay and should be retested in duplicate before interpretation.
- 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 assay.
- Initially reactive specimens, which are negative in both wells on the repeat test, are considered negative for antibodies to HIV-1 and HIV-2.
- Specimens, which are repeatedly positive in this assay, should be further tested by additional more specific tests.

### Limitations

Repeatedly reactive results with Biotec's HIV 1&2 kit are presumptive evidence of the specimen containing antibodies. 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 Biotec HIV 1&2 assay 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. This kit does not detect HIV-1 subgroup O.

## PERFORMANCE CHARACTERISTICS

**Specificity: 99.92%** (refer to table below).

Sample	N	Positive	Negative
Normal donor serum/plasma	10274	8	10266
HBsAg positive sample	100	0	100
Anti-HCV positive sample	84	0	84
Anti- <i>T. pallidum</i> positive sample	63	0	63

**Sensitivity: 100%** (not including indeterminate sample – refer to table below).

Sample	N	Positive	Negative
Anti-HIV-1 positive serum	512	512	0
Anti-HIV-1 low titre serum	5	5	0
Anti-HIV-2 positive serum	3	3	0
Indeterminate	8	8	3

**Coefficient of variation (CV) = 4.75%** (n = 16 for each of 4 lots).