



## HTLV BLOT 2.4

### WESTERN BLOT ASSAY

FOR RESEARCH USE ONLY  
NOT FOR USE IN  
DIAGNOSTIC PROCEDURES

REVISION DATE: 11/05  
MAK 0012-ENG-0

Note Changes Highlighted

For detection of antibodies to HTLV-I and HTLV-II in serum or plasma samples.

#### NAME AND INTENDED USE

The **MPD Diagnostics (MPD) HTLV BLOT 2.4** is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to HTLV-I and HTLV-II in human serum or plasma samples. This test kit is supplied for research purposes only. It is not intended for use in the diagnosis or prognosis of disease. In particular, this test cannot be used to evaluate blood specimens for the purposes of donor screening or as a confirmatory diagnostic.

#### INTRODUCTION

The **MPD HTLV BLOT 2.4** is an informational research test on serum or plasma samples. The **MPD HTLV BLOT 2.4** incorporates MTA-1, a unique HTLV-I envelope recombinant protein (rgp46-1), K55, a unique HTLV-II envelope recombinant protein rgp 46-II and GD21, a common yet specific HTLV-I and HTLV-II epitope recombinant envelope protein. Each strip also includes an internal sample addition control to minimize the risk of false negatives due to operational errors.

#### CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips are incorporated with HTLV-I viral proteins derived from native inactivated disrupted viral particles and genetically engineered proteins. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and controls. Specific antibodies to HTLV-I/II, if present in the sample will bind to the HTLV-I/II proteins on the strips. The strips are washed to remove unbound materials while antibodies that bind specifically to the HTLV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate, BCIP/NBT.

#### KIT COMPONENTS

- |   |  |
|---|--|
| 1. <b>NITROCELLULOSE STRIPS</b><br>Incorporated with HTLV-I viral lysate and recombinant envelope antigens. Keep dry and away from light.   | Available<br>in 18 or 36<br>strips                           |
| 2. <b>NON-REACTIVE CONTROL</b><br>Inactivated normal human serum non-reactive for anti-HCV, anti-HIV-1/2, anti-HTLV-I/II and HBsAg. Contains sodium azide and thimerosal as preservatives.                            | 1 vial<br>(80 µl)  |
| 3. <b>STRONG REACTIVE CONTROL I</b><br>Inactivated human serum with high titered antibodies to HTLV-I and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives.   | 1 vial<br>(80 µl)  |
| 4. <b>STRONG REACTIVE CONTROL II</b><br>Inactivated human serum with high titered antibodies to HTLV-II and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives. | 1 vial<br>(80 µl)  |
| 5. <b>LYOPHILIZED STOCK BUFFER</b><br>To be reconstituted in reagent grade water. Tris buffer with heat inactivated animal and non-animal proteins. Contains thimerosal as preservative.                              | 1 or 2 bottles<br>(each to be<br>reconstituted<br>to 100 ml) |
| 6. <b>WASH BUFFER CONCENTRATE (20X)</b><br>Tris with Tween-20 and contains thimerosal as preservative.  | 1 bottle<br>(70 ml)  |
| 7. <b>CONJUGATE</b><br>Goat anti-human IgG conjugated with alkaline phosphatase.  | 1 vial<br>(120 µl)   |
| 8. <b>SUBSTRATE</b><br>Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).   | 1 bottle<br>(100 ml)   |
| 9. <b>BLOTTING POWDER</b><br>Non-fat dry milk   | 10 sachets<br>(1g each)                                      |
| 10. Incubation trays, 9 wells each.   | 2 or 4 trays   |
| 11. Instruction Manual  | 1 copy   |
| 12. Forceps   | 1 pair   |

Volume of reagents provided are sufficient for 4 runs.

#### PRECAUTIONS TO USERS

**CAUTION:** Handle all assay specimens, positive and negative controls as potentially infectious agents.

1. Substituting reagents, even between lots, may affect results.
2. FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
3. Do not use kit components beyond the expiry date.
4. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
5. Gloves and lab coats must be worn.
6. Do not pipette by mouth.
7. Wipe spills quickly and thoroughly with sodium hypochlorite solution.
8. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal.
9. It is highly recommended that this assay be performed in a biohazard cabinet.
10. Decontaminate all used chemicals and reagents in sodium hypochlorite solution.
11. We do not recommend re-use of incubation trays.

#### STORAGE INSTRUCTIONS

- A. **Antigen strips**
- Avoid unnecessary exposure of antigen strips to light.
- B. **Reagents**
- Store all reagents at 2-8°C.
  - For best results, dispense all reagents while cold and return to 2-8°C storage as soon as possible.

**CAUTION:** Avoid unnecessary exposure of substrate to light.

#### MATERIALS REQUIRED BUT NOT PROVIDED

Rocking platform \*  
Pipettor and tips  
Aspirator with sodium hypochlorite trap \*  
56°C water bath (optional)

\* Not required if using **Autoblot System 20**.

#### SPECIMEN HANDLING AND STORAGE (OPTIONAL)

Sera can be inactivated but this is not a requirement for optimal test performance.

inactivated as follows:

1. Loosen caps of serum containers.
2. Heat serum at 56°C for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis.

We recommend that the sera should not undergo repeated freeze-thaw cycles prior to testing.

#### PREPARATION OF REAGENTS

1. **DILUTED WASH BUFFER**
  - (a) Dilute 1 volume of WASH BUFFER CONCENTRATE (20X) with 19 volumes reagent grade water. Mix well.
2. **BLOTTING BUFFER**
  - (a) Reconstitute each bottle of LYOPHILIZED STOCK BUFFER with 100ml reagent grade water. Mix well to dissolve. This RECONSTITUTED STOCK BUFFER is stable for 6 weeks if stored at 2-8°C
  - (b) BLOTTING BUFFER should be prepared fresh prior to use.  
Add 1 g of BLOTTING POWDER to every 20 ml of the RECONSTITUTED STOCK BUFFER prepared in step 2(a) above. Mix well.
3. **WORKING CONJUGATE SOLUTION**
  - (a) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example 10µl CONJUGATE to 10ml BLOTTING BUFFER.
  - (b) WORKING CONJUGATE SOLUTION should be prepared fresh prior to use.
4. **SUBSTRATE SOLUTION (ready to use)**
  - (a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

### RECOMMENDED ASSAY PROCEDURE

Note: a) Aspirate all used chemicals and reagents into a trap containing Sodium hypochlorite.

b) All incubations are to be carried out on a rocking platform.

#### Caution:

Some samples cause dark patches on the spot of the strip where they are added. To avoid this problem, one should ensure the following:-

- i. Sample should be added only after BLOTTING BUFFER is added.
- ii. Tilt the tray slightly by elevating either the top or bottom end of the tray. The Blotting Buffer will flow to the lower end of the tray. Add the sample where the Blotting Buffer is collected. When all the samples are added, return the tray back to its original flat position. Always ensure that the strips are kept wet during the process.
- iii. Alternatively, if tilting the tray is not desired, the samples may be added to the top or bottom end of the well. This way if dark patches showed, the reading of the strip results will not be affected.

#### Procedure:

1. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive, Weak Reactive and Non-Reactive controls.
2. Add 2 ml of DILUTED WASH BUFFER to each well. 2 ml
3. Incubate the strips for at least 5 minutes at room temperature ( $25 \pm 3^\circ\text{C}$ ) on a rocking platform (speed of 12 to 16 oscillations per minute). Remove buffer by aspiration. 5 minutes
4. Add 2 ml of BLOTTING BUFFER to each well. 2 ml
5. Add 20  $\mu\text{l}$  each of sera or controls to appropriate wells. 20  $\mu\text{l}$
6. Cover the tray with the cover provided and incubate for 1 hour at room temperature ( $25 \pm 3^\circ\text{C}$ ) on the rocking platform. 60 minutes
7. Carefully uncover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.
8. Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash. 3 x 2 ml

9. Add 2 ml of WORKING CONJUGATE SOLUTION to each well. 2 ml
10. Cover tray and incubate for 1 hour at room temperature ( $25 \pm 3^\circ\text{C}$ ) on the rocking platform. 60 minutes
11. Aspirate CONJUGATE from the wells. Wash as in step 8. 3 x 2 ml
12. Add 2 ml of SUBSTRATE SOLUTION to each well. 2 ml
13. Cover tray and incubate for 15 minutes on the rocking platform. 15 minutes
14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction. 3 x 2 ml
15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.
16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands (See Interpretation of Results) and grade the results. For storage, keep the strips in the dark.

### AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED							
	3	6	9	15	20	27	36	
1X Wash Buffer (ml)	60	100	140	240	300	400	520	
1X Blotting Buffer (ml)	20	40	60	80	100	120	160	
Conjugate ( $\mu\text{l}$ )	11	17	23	35	45	59	77	
Substrate (ml)	11	17	23	35	45	59	77	
Blotting Powder (g)	1	2	3	4	5	6	8	

### REFERENCE STANDARDS

We recommend that the Non-Reactive Control and both Strong Reactive Controls be run with assay regardless of the number of samples tested.

1. **NON-REACTIVE CONTROL**  
No HTLV-I/II viral specific bands, rpg46-I, rpg 46-II or GD21 should be observed on the Non-Reactive control strip. The band for the serum control (anti-human IgG) should be visible.
2. **STRONG REACTIVE CONTROL I**  
The serum control band and all relevant HTLV-I/II molecular weight bands must be evident. The relevant HTLV-I bands must be present are p19, p24, gp46, gp46-1 and GD21.
3. **STRONG REACTIVE CONTROL II**  
The serum control band and all relevant HTLV-I/II molecular weight bands must be evident. The relevant HTLV bands must be present are p24, GD21 and rpg46-II.

#### IDENTIFICATION OF BANDS

The serum control band serves as a check for serum addition in the assay. Absence of this band indicates that no test serum or conjugate or substrate has been dispensed onto the test strip or other operational errors.

Locate and identify bands on the strips run with Strong Reactive Controls. These strips are then used to identify bands present on strips used with test specimens.

Serum with antibodies to both viruses although rare, may occur and can also be differentiated based on the above criteria. Banding patterns of such specimens will indicate HTLV-I and HTLV-II positive. Available data demonstrates that the seroreactivity to rgp46-I is specific for HTLV-I and seroreactivity to rgp46-II is specific for HTLV-II.

#### LIMITATIONS OF THE PROCEDURE

Deviation from the recommended procedure may lead to aberrant results.

#### LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss however caused by the product in the use or in the application thereof.

#### REFERENCES

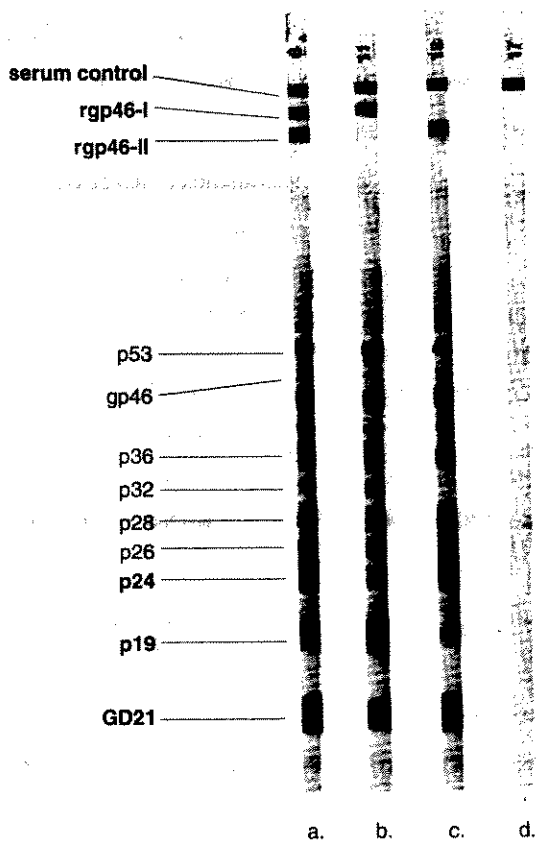
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#### Manufacturer:

**MP Biomedicals Asia Pacific Pte Ltd.**  
85 Science Park Drive  
#04-01, The Cavendish  
Singapore Science Park  
Singapore 118259  
Tel. No. : + 65 6775 0008  
Fax. No. : + 65 6775 4536  
Email : enquiry\_ap@mpbio.com

FIGURE 1



Viral specific bands as visualized with:  
a. Serum Reactive to both HTLV-I and HTLV-II  
b. Strong Reactive Control I. (Reactive for HTLV-I only)  
c. Strong Reactive Control II. (Reactive for HTLV-II only)  
d. Non-reactive Control.

## TROUBLE SHOOTING CHART

