HTLV BLOT 2.4

WESTERN BLOT ASSAY

Instructions For Use

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

REVISION DATE: 09/10
MAK0012-ENG-3

Note Changes Highlighted

REF (18 tests kit) : 11082-018
(36 tests kit) : 11082-036

NAME AND INTENDED USE

The **MP Diagnostics 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 Use Only. It is not intended for use in the diagnosis or prognosis of disease.

INTRODUCTION

The **HTLV Blot 2.4** is intended as a supplemental antibody assay for Research Use Only. The possible serological profiles defined by the **HTLV Blot 2.4** include the following: HTLV Seropositive, HTLV-I Seropositive, HTLV-II Seropositive, Seronegative or Indeterminate.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on **MP Diagnostics** products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. Some of the common symbols are explained in more detail in the British and European Standard BS EN 980: 2008.

Use by
Synonyms for this: Expire Date

Batch Code
Synonyms for this are:
Lot Number
Batch Number

Temperature Limitation

Manufacturer

Contains sufficient for <n> tests

Consult Instructions for Use

Catalogue Number

Caution

Do not reuse

Harmful (Xn) / Irritant (Xi)

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 specimen 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

	Component Description	Quantity Provided
ANTIGEN STRIPS	**NITROCELLULOSE STRIPS** Incorporated with HTLV-I viral lysate and recombinant envelope antigens, and a serum addition control (anti-human IgG) band. Keep dry and away from light.	Available in 18 or 36 strips
CONTROL —	**NON-REACTIVE CONTROL** 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 (80 µl)
CONTROL I +	**STRONG REACTIVE CONTROL I** 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 (80 µl)
CONTROL II +	**STRONG REACTIVE CONTROL II** 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 (80 µl)
BUF LYO. STOCK	**LYOPHILIZED STOCK BUFFER** 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 (each to be reconstituted to 100 ml)
BUF WASH 20x	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide as a perservative.	1 vial (160 µl)

SUBS BCIP / NBT	**SUBSTRATE** Solution of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT).	1 bottle (100 ml)
POWDER BLOTTING	**BLOTTING POWDER** Non-fat dry milk	10 packets (1g each)
	Incubation Tray, 9 wells each	2 or 4 trays
	Instructions For Use	1 copy
	Forceps	1 pair

Note : Volume of reagents provided are sufficient for 4 runs.

WARNINGS AND PRECAUTIONS

1. For *Research Use Only*. It is not intended for use in the diagnosis or prognosis of disease.
2. For Professional use only.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection.

HANDLE ASSAY SPECIMENS, STRONG REACTIVE AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS. It is recommended that the components and test specimens be handled using universal precautions. They should be disposed of in accordance with established safety procedures.

The ***Strong Reactive Control I, Strong Reactive Control II*** and ***Non-Reactive Control*** contain Thimerosal and Sodium Azide, the Stock Buffer Concentrate and Wash Buffer Concentrate contain Thimerosal and the Conjugate contains Sodium azide. Sodium azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system.

STORAGE

1. Store **HTLV BLOT 2.4** kit and its components at 2°C to 8°C when not in use.

2. All test reagents and strips when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze reagents.

A. **Antigen strips**
- Avoid unnecessary exposure of antigen strips to light.

B. **Reagents**
- Store reagents in their original vials or bottles, and they should be closed for storage.
- Dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
- Precipitates may form when the Substrate is stored at 2°C to 8°C. This will not affect the performance of the kit.

CAUTION: Avoid unnecessary exposure of substrate to light.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Serum or plasma samples collected in EDTA, heparin or sodium citrate may be used. Before storage, ensure that blood clot or blood cells have been separated by centrifugation.

Samples should be stored at 2°C to 8°C if the test is to be run within 7 days of collection or frozen at -20°C or colder if the test is to be delayed for more than 7 days. Clear, non-hemolyzed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

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.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Optimal assay performance requires STRICT ADHERENCE to the assay procedure described below. Deviations in procedure or equipment may produce aberrant results.

1. Deionized or distilled water, reagent grade
2. Disposable gloves
3. Rocking platform (designed with a rocking speed of 12 to 16 oscillations per minute and which moves through a 5° to 10° tilt to wash membranes evenly)
4. Pipettors and tips of appropriate volume
5. Aspirator with sodium hypochlorite trap
6. 56°C water bath (optional)
7. Sodium hypochlorite for decontamination

PREPARATION OF REAGENTS

1. DILUTED WASH BUFFER
(a) DILUTED WASH BUFFER should be **prepared fresh prior to use.**
(b) Dilute 1 volume of WASH BUFFER CONCENTRATE (20x) with 19 volumes of 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°C-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. Stir to ensure powder dissolves completely.
(c) Stir again before dispensing.

3. WORKING CONJUGATE SOLUTION
Note : Prepare solution in polypropylene container / beaker.
(a) WORKING CONJUGATE SOLUTION should be **prepared fresh prior to use.**
(b) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example 10µl CONJUGATE to 10ml BLOTTING BUFFER.

4. SUBSTRATE SOLUTION (ready to use)
(a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

ASSAY PROCEDURE - Manual Use Only

NOTE: - The procedures identified in this Instructions for Use document are for manual testing only.

Note: a) Aspirate all used chemicals and reagents into 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 2ml of DILUTED WASH BUFFER to each well.	**2 ml**
3.	Incubate the strips for at least 5 minutes at room temperature (25°C ± 3°C) on a rocking platform (speed of 12 to 16 oscillations per minute). Remove buffer by aspiration.	**5 minutes**
4.	Add 2ml of BLOTTING BUFFER to each well.	**2 ml**
5.	Add 20µl each of sera or controls to appropriate wells.	**20 µl**
6.	Cover the tray with the cover provided and incubate for 1 hour at room temperature (25°C ± 3°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 2ml 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°C ± 3°C) on the rocking platform.	**60 minutes**
11.	Aspirate WORKING CONJUGATE SOLUTION 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 several 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 and record the result. For storage, keep the strips in the dark.	

SUMMARY OF ASSAY PROTOCOLS

Reagents	Qty	Duration
Nitrocellulose strip	1	-
Wash Buffer	2 ml	5 mins
Blotting Buffer	2 ml	-
Specimen	20µl	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Conjugate	2 ml	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Substrate (Ready to use)	2 ml	15 mins
Distilled Water	3 x 2 ml	-

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 (µl)	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

QUALITY CONTROL

We recommend that the Non-Reactive Control and both Strong Reactive Controls be run with assay regardless of the number of samples tested. In order for the results obtained from any assay to be considered valid, the following conditions must be met:

1. NON-REACTIVE CONTROL
No HTLV-I/II viral specific bands, rpg46-I, rpg46-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 that must be present are p19, p24, gp46, rgp46-I and GD21. Note that the gp46 band is diffused. The serum control (anti-human IgG) band should be visible.

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 that must be present are p24, GD21 and rpg46-II. The serum control (anti-human IgG) band should be visible.

LIMITATIONS OF THE PROCEDURE

Optimal assay performance requires the strict adherence to the assay procedure described. 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 Instructions For Use when used in accordance with the instruction contained therein. The manufacturer disclaims any warranty express or implied, including such express or implied warranty with respect to merchantability, fitness for use or implied utility for any other purpose. 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 howsoever caused by the product in the use or in the application thereof. The manufacturer makes no representation express or implied, that this product will not infringe the intellectual property rights of the third parties.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be a technical problem / complaint, please do the following:
1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

REFERENCES

1. Towbin H., Staehlin T. and Gordan J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 1976; 76 4350-4354.

2. Poiesz BJ., Ruscetti FW., Gazdar AF., Bonn PA., Minna JD. And Gallo RC. Detection and Isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. U.S.A. 1980; 77(12): 7415-7419.

3. Kalyanaraman VS., Sarngadharan MG., Robert-Guroff M., Miyoshi I., Blayney D., Golde and Gallo RC. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 1982; 218: 571-573.

4. William AE., Fang CT., Slamon DJ. et al. Seroprevalence and epidemiological correlates of HTLV-I infection in U.S. blood donors. Science 1988; 240: 643-646.

5. Lee H., Swanson P., Shorty VS., Zack JA., Roseblatt JD. and Chen ISY. High rate of HTLV-II infection in seropositive IV drug abusers in New Orleans. Science 1989; 244: 471-475.

6. Lipka JJ., Bui K., Reyes GR, Moeckli R., Wiktor SZ., Blattner WA., Murphy EL., Hanson CV., Shaw GM., Shinsky JJ. and Foung SKH. Determination of a unique immunodominant epitope of HTLV-I. Infect Dis 1990; 162: 353-357.

7. Wiktor SZ., Alexandra SS., Shaw GM. et al. Distinguishing between HTLV-I and HTLV-II by Western Blot. Lancet 1990; 335: 1533.

8. Samuel KP., Lautenberger JA., Jorcyk CL., Josephs S., Wong Staal F. and Papas TS. Diagnostic potential for human malignancies of bacterially produced HTLV-I enevelope protein. Science 1984; 226: 1094-1097.

9. Hadlock KG., Goh CJ., Bradshaw PA., Perkins S., Lo J., Habbaz RK., Kaplan J. and Foung SKH. Delineation of an immunodominant and highly HTLV specific epitope within the HTLV-I transmembrane glycoprotein. Blood 1995; 68(4): 1392-1399.

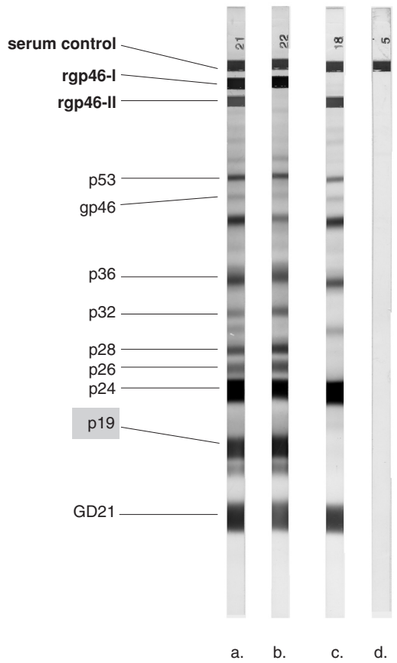
10. Varma M., Rudolph D., Knuchel M., Switzer W., Hadlock KG., Velligan M., Chan L., Foung SKH., Lal RB. Enhanced specificity of truncated transmembrane protein for serologic confirmation of HTLV-I and HTLV-II infection by Western Blot assay containing recombinant envelope glycoproteins. J. Clin. Micro. 1995; 33(12): 3239-3244.

Manufacturer:

MP Biomedicals Asia Pacific Pte Ltd
2 Pioneer Place
Singapore 627885

Tel. No. : +65 775 0008
Fax No. : +65 774 6146
Email: enquiry_ap@mpbio.com

FIGURE 1



Viral specific bands as visualized with:
a. A HTLV-I/II dual infection serum.
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

Dark spots develop on strips

1. Bacterial or fungal contamination of test sample.
2. Precipitation of immune complexes in aged test sample.
3. Bacterial or fungal contamination on strip due to improper storage.
4. Strips physically damaged, cracked or scratched.
5. Strips not properly washed between assay steps.

White patches develop on strips

1. Strips flipped over during assay.
2. Trays not properly washed before use.
3. Poor dissolution of Blotting Powder.
4. Electrotransblot interference during manufacturing.

Expected bands do not develop or are of weak intensity.

Check positive control

- **Positive control weak**

  The problem is probably caused by the reagents.

  1. Reagents not properly prepared.
  2. Wrong conjugate dilution.
  3. Unstable reagents due to improper temperature exposure.
  4. Conjugate contaminated with human IgG.
  5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
  6. Trays, reagent(s) or water having high phosphate concentration.
  7. Rotary platform used instead of Rocking platform.

- **Positive control OK**

  The problem is probably caused by test sample.

  1. Wrong test sample dilution.
  2. Test sample contaminated with conjugate.
  3. Test samples severely immune-complexed.
  4. Test sample IgG deteriorated or denatured due to repeated freeze- thaw or improper storage.
  5. Rotary platform used instead of Rocking platform.
  6. Test sample may be an ELISA “false” positive.

Strong Background develops on strip in the absence or presence of positive bands.

1. overdeveloped strips (stop reaction sooner).
2. Incomplete washing.

Absence of Serum Control Band

1. Serum not added.
2. Strips flipped over during assay.
3. Conjugate not added.
4. Substrate not added.

Bands other than the Serum Control band develops on negative control

Tray wells or Control may have been crossed contaminated.

Non-specific bands and/or dark background develop on strips

1. Wrong test sample or conjugate dilution.
2. Test sample/reagent incubation too long.
3. Incomplete washing during assay.
4. Incubation temperature greater than 30˚C.
5. Test sample reactive with non-viral proteins.

Strips are defective

1. They are cracked.
2. They contain air bubbles which cause the appearrance of white spots in reactive zones big enough to prevent any detection.
3. They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user's site.

HTLV-specific bands detected on negative control and/or known negative samples

1. Trays contaminated with positive test sample or positive control.
2. Negative control/test sample contaminated with positive control/ test sample.
3. Same pipette tip used for delivery and/or removal of, test samples/control.
4. Test sample may be an ELISA “false” negative.

Watery marks on developed strips

1. Strips left to dry after pre-soaking step prior to adding Blotting Buffer.