HTLV I/II ELISA 4.0

Instructions For Use

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

REVISION DATE: 09/08 Note: Changes Highlighted
MBG 0012-ENG-1

23082-192: (192 tests kit)
23082-480: (480 tests kit)

NAME AND INTENDED USE

The **MP Diagnostics (MPD) HTLV I/II ELISA 4.0** is an enzyme-linked immunosorbent assay intended for the detection of antibodies to Human T-cell Lymphotropic Virus type 1 (HTLV-I) and type 2 (HTLV-II) in human serum or plasma.

This kit is supplied for research purposes only. It is not intended for use in the diagnosis or prognosis of disease.

INTRODUCTION

Human T-cell Lymphotropic Viruses (HTLVs) are pathogenic retroviruses that may cause severe haematological and neurological diseases in infected individuals. The HTLV family comprises of two well-studied members: HTLV-I and HTLV-II, as well as two newly discovered members: HTLV-3 and HTLV-4. HTLV-I is known as the etiological agent of adult T-cell leukemia / lymphoma (ATL), HTLV-associated myelopathy / tropical spastic paraparesis (HAM/TSP), and HTLV-associated uveitis. HTLV-II infection has also been associated with leukemia and neurological disease although it is less pathogenic than HTLV-I. Several lines of molecular evidences suggest that HTLV-3 possesses some of the HTLV-I properties although little is known about the pathogenicity of HTLV-3.

Studies of the geographic distribution of HTLV-I infection reveal that the virus is highly prevalent in Japan, Africa, Caribbean islands and South America. Recent epidemiological studies in the United States and Europe confirm the presence of a mixed prevalence of both HTLV-I and HTLV-II among different high-risk populations, such as intravenous drug users and transfusion recipients. The viruses can be transmitted through sexual contact, and through contaminated blood products, and mother to child via breast-feeding.

The **MPD HTLV I/II ELISA 4.0** is a direct sandwich immunoassay that utilizes a combination of recombinant proteins and a tri-fusion recombinant protein labeled with horseradish peroxidase. This test format assures simultaneous detection of various specific IgA, IgG and IgM antibodies against HTLV-I and HTLV-II. In addition, external tests have shown that **MPD HTLV I/II ELISA 4.0** is capable of detecting antibodies to HTLV-3/STLV-3 (see section on “Specific Performance Characteristics”).

The **MPD HTLV I/II ELISA 4.0** is intended as a semi-qualitative enzyme-linked immunosorbent assay for the detection of antibodies to both HTLV-I and HTLV-II found in human serum or plasma.

CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The wells of the polystyrene microplate strips are coated with a mixture of three different HTLV recombinant proteins, which correspond to the highly antigenic segments of HTLV-I and HTLV-II viruses. The conjugate is based on a tri-fusion recombinant protein, which is labeled with horseradish peroxidase. The tri-fusion antigen is generated by cloning of three cDNA fragments coding for the three HTLV recombinant proteins into a single vector. Human serum or plasma, diluted in the diluent containing the conjugate, is incubated in these coated wells. HTLV-I/II specific antibodies (IgA, IgG and IgM), if present, will bind to both the antigens immobilised on the solid phase and the tri-fusion antigen of the conjugate. After incubation, the wells are thoroughly washed to remove unbound materials A colorless substrate solution containing chromogen 3,3’,5,5’ – tetramethylbenzidine (TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of a blue colour after incubation, which changes to yellow when the color reaction is terminated by the addition of sulphuric acid. The intensity of the resulting yellow product is measured at 450nm using a spectrophotometer and is proportional to the amount of antibodies present in the specimen.

KIT COMPONENTS

	Component Description	Quantity Provided	
1.	**HTLV MICROPLATE** Twelve 8-well strips per plate. Each microplate well contains adsorbed HTLV-I and HTLV-II recombinant proteins. **Content :** 96 wells per plate **Storage :** 2°C to 8°C.	2 PLATES 192 tests	5 PLATES 480 tests
2.	**NON-REACTIVE CONTROL** Normal human serum, non-reactive for anti-HCV, anti-HIV-1/2, anti HTLV-I/II and HBsAg. Contains thimerosal and sodium azide as preservatives. **Contents**: 1.2 ml per vial **Storage :** 2°C to 8°C.	1 vial	2 vials
3.	**REACTIVE CONTROL** Inactivated human serum containing a high titer of IgG antibodies specific for HTLV. Contains thimerosal and sodium azide as preservatives. **Contents**: 1.2 ml per vial **Storage :** 2°C to 8°C.	1 vial	2 vials

4. **DILUENT** — 1 bottle — 2 bottles
Phosphate-buffered saline solution containing casein and detergent. Contains Bronidox™ as preservative.
Contents: 50 ml per bottle.
Storage : 2°C to 8°C.

5. **PLATE WASH CONCENTRATE (20X)** — 1 bottle — 2 bottles
Phosphate buffered saline with Tween-20. Contains chloroacetamide as preservative.
Contents: 120 ml per bottle.
Storage : 2°C to 8°C..

6. **CONJUGATE** — 1 vial — 2 vials
HTLV tri-fusion antigen labeled with horseradish peroxidase. Contains 0.02% thimerosal as preservative.
Contents: 160 µl per vial
Storage : 2°C to 8°C.

7. **SUBSTRATE** — 1 bottle — 3 bottles
Colorless solution containing 3,3',5,5' Tetramethylbenzidine (TMB).
Contents: 25 ml per bottle
Storage : 2°C to 8°C in the dark

8. **PLATE COVERS** — 8 pieces — 12 pieces
Adhesive covers for microplate during incubation.

9. **INSTRUCTIONS FOR USE** — 1 copy

Note : STOP SOLUTION (2M H_2SO_4) is not provided in the kit. For preparation protocol, please refer to section <**PREPARATION OF REAGENTS**>**.**

WARNINGS AND PRECAUTIONS

This kit is for research use only.

1. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
2. Do not pipette by mouth.
3. Handle assay specimens, microplates, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
5. It is highly recommended that this assay be performed in a biohazard cabinet.
6. Keep materials away from food and drink.
7. In case of an accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
8. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
9. Sulphuric acid can cause burns. **AVOID CONTACT**. If it comes into contact with skin, wash thoroughly with water.
10. Avoid contact of Sulphuric acid with any oxidizing agent or metal.
11. Do not expose substrate to strong light.
12. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with an effective disinfecting agent before work is resumed.

ANALYTICAL PRECAUTIONS

1. Use only sera or plasma samples collected in EDTA, Heparin, Sodium Citrate, K-Oxalate or Acid Citrate Dextrose (ACD). Before storage, ensure that blood clot or blood cells have been separated by centrifugation.
2. Do not use whole blood or other body fluids
3. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this IFU. Deviations from the procedure may lead to aberrant results.
4. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and microplates are matched for optimal performance. Use only the reagents supplied with the kit.
5. Do not use kit components beyond the expiry date printed on the kit box.
6. Avoid microbial contamination of the reagents, when opening and removing aliquots from the original vials or bottles. As this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.
7. To prevent cross contamination, use a new pipette tip for each specimen aliquoted to, and do not touch the top or the bottom of the strips, the edge of the wells or the liquid in the wells with fingers or pipette tips.
8. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.
9. For best results allow all reagents and samples to reach room temperature (25ºC ± 5ºC) before use. Immediately after use return to 2ºC to 8ºC storage.
10. Use only reagent grade quality, deionised or distilled water to dilute reagents.
11. ALL REAGENTS MUST BE MIXED WELL BEFORE USE.

12. WORKING CONJUGATE SOLUTION SHOULD BE PREPARED FRESH PRIOR TO USE.

13. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.

14. Do not remove microplates from the storage bag until immediately before use. Opened, unused strips should be stored at 2ºC to 8°C in its storage bag with the desiccant provided.

15. The kit controls should be assayed concurrently with patients' samples for each test run.

16. Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not "blow out' from the micropipette. It is recommended to use reverse pipetting whenever possible.

17. Use of highly haemolyzed samples, incomplete clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.

18. **DO NOT USE A WATER BATH TO INCUBATE MICROPLATES.**

19. During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.

20. Avoid repeatedly opening and closing the incubator door during incubation steps.

21. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Remove any bubbles in the well, e.g. by gentle tapping.

22. Ensure that automated equipment if used is validated before use.

23. Routine maintenance of aspiration / wash system is strongly recommended to prevent carryover from highly reactive specimens to non-reactive specimens.

STORAGE

1. Store **MPD HTLV I/II ELISA 4.0** kit and its components at 2ºC to 8ºC when not in use.

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

3. Crystals may form when Plate Wash Concentrate (20x) is stored at 2ºC to 8ºC. These crystals must be dissolved by warming at 37°C prior to use.

4. The stability of the kit after first opening is 12 months. Kit expiry will be the earliest expiry date either in the **closed** or **opened** condition.

5. Opened, unused microplate strips must be stored with the desiccant provided at 2ºC to 8ºC in a closed pouch.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

Fresh specimens are preferred, specimens that undergo freeze-thaw cycles repeatedly are not recommended. Specimens should be stored 2ºC to 8ºC if the test is to be run within 7 days of collection or frozen at ≤ -20ºC if the test is to be delayed for more than 7 days. In addition, up to 0.1% sodium azide may be used to stabilize serum or plasma specimens stored at 2ºC to 8ºC.

Clear, non-haemolysed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

Specimens can be virus inactivated, although it might not be optimal for test performance as potential effect of the treatment on IgM antibody is not fully understood. If necessary, inactivate as follows:

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

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable absorbent bench top paper and paper towels.
2. Polypropylene tubes or containers.
3. Graduated pipettes: 5 ml, 10 ml.
4. Multichannel pipettor capable of delivering 50 µl, 100 µl, and 200 µl.
5. Pipettor capable of delivering 1-1000 µl.
6. Disposable pipette tips.
7. Reagent reservoirs (troughs) with a capacity of 25 ml.
8. Deionised or distilled water, reagent-grade quality.
9. Flasks: 500 ml, 1 litre.
10. 2M sulphuric acid (as stop solution), 6 ml is required per plate
11. ELISA Microplate Washer. Alternatively, washing can be performed manually by using a multichannel pipettor delivering 0.3ml volumes and an aspirator device.
12. A 37 ± 1ºC incubator.
13. A dual (A_{450}–A_{620}) or single (A_{450}) wavelength microassay plate reader.
14. Effective disinfectant.

PREPARATION OF REAGENTS

1. WORKING CONJUGATE

a. **WORKING CONJUGATE** should be **prepared fresh prior to use**.
b. Mix **CONJUGATE** and **DILUENT** thoroughly before use. **DO NOT SPIN** the conjugate vial.
c. Dilute **CONJUGATE** at 1:200 dilution factor with **DILUENT**. For example, add 10 µl conjugate into 2.0 ml diluent.
d. Use only polypropylene containers or tubes.
e. 6.0 ml of **WORKING CONJUGATE** is required for one microplate.

CONJUGATE PREPARATION CHART		
Number of tests	Vol. of Conjugate (μl)	Vol. of Diluent (ml)
24	15	3.0
48	25	5.0
72	30	6.0
96	40	8.0

2. DILUTED PLATE WASH (1X PLATE WASH)

a. **DILUTED PLATE WASH (1X PLATE WASH)** is stable for 2 weeks at room temperature**.**

b. Dilute 1 volume of **20X PLATE WASH** with 19 volumes of distilled water (reagent grade quality). Mix well. Approximately 200 ml of wash buffer is required to wash 1 plate.

3. STOP SOLUTION, 2M H_2SO_4 (not provided in the kit, 6ml is needed per plate)

a. Add about 11.2 ml of analytical grade concentrated sulphuric acid slowly to 80 ml of distilled or dionized water **(caution: NOT in reverse order)** and then top up to 100 ml with more water.

ASSAY PROCEDURE

IMPORTANT:- Immunoassays of this nature are temperature-sensitive and time-dependent. Strict adherence to the assay procedure will ensure optimal assay performance. Deviations from the recommended procedure may lead to aberrant results.

1.	Equilibrate all kit components and test specimens to room temperature before use.	**-**
2.	Prepare **WORKING CONJUGATE** as described in the **PREPARATION OF REAGENTS**.	**-**
3.	Remove one microplate from aluminum pouch.	**-**
4.	Mix specimen and control vials thoroughly before use.	**-**
5.	Fill a reagent reservoir with **WORKING CONJUGATE.** Using a multichannel pipettor, add 50 μl of **WORKING CONJUGATE** to all wells.	**50 μl**
6.	Wells A1 and B1 are '**BLANKS'. DO NOT ADD SPECIMEN TO THESE WELLS**. Add 50 μl of diluent per well to these wells.	**50 μl**
7.	Add 50 μl of test specimen to the assigned well, starting at well A2. This will give a final specimen dilution of 1:2. **Mix by pipetting up and down at least once.** Repeat this step with other test specimens until all is added.	**50 μl**
8.	Add 50 μl of **NON-REACTIVE CONTROL** per well to wells C1, D1 and E1. **Mix by pipetting up and down at least once.**	**50 μl**
9.	Add 50 μl of **REACTIVE CONTROL** per well to wells F1, G1 and H1. **Mix by pipetting up and down at least once.**	**50 μl**
10.	Tap gently on all sides of the microplate to ensure proper mixing of the specimens and controls. Carefully cover the microplate with a Plate Cover to prevent evaporation during incubation.	**-**
11.	**Incubate for 60±2 minutes at 37±1°C in an incubator (Do not use a waterbath for incubation).**	**60±2 min 37±1°C**
12.	Remove and discard the plate cover and wash the microplate with **DILUTED PLATE WASH (1X PLATE WASH)** using one of the two recommended methods:- A. Automated or Semi-automatic Microplate Washer - Wash six (6) times with at least 300 μl per well per wash. B. Manual Microplate Washer – Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. **BE CAREFUL NOT TO SCRATCH THE INSIDE OF THE WELL SURFACE**. Fill the entire plate with at least 300 μl/well, then aspirate immediately in the same order. Perform this cycle six (6) times.	**300 μl per well per wash**
13.	Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry. Colour formation can be inhibited during the substrate incubation by residual plate wash buffer.	**-**
14.	Fill a reagent reservoir with **SUBSTRATE**. Using a multichannel pipettor, add 100 μl of **SUBSTRATE** to each well. Apply a Plate Cover.	**100 μl**
15.	**Incubate for 30±2 minutes in the dark at 37±1°C.**	**30±2 min 37±1°C**
16.	Remove and discard the Plate Cover.	**-**
17.	Using a multichannel pipettor, add 50 μl of **2M Sulphuric Acid** to each well to stop the color reaction. Tap gently to mix the plate.	**50 μl**
18.	Determine the absorbance for each well at 450 nm. If a dual filter instrument is used, the reference wavelength should be 620 nm.	**$A_{450/620\ nm}$**

NOTE: Absorbance should be read within 10 minutes upon addition of the 2M H_2SO_4 STOP SOLUTION.

QUALITY CONTROL

1. **Please ensure that each test specimen and control is properly mixed with WORKING CONJUGATE by pipetting up and down at least once after addition.**
2. **Change in color of WORKING CONJUGATE indicates that serum or plasma has been added.**
3. The BLANK should be assayed in duplicate, whereas NON-REACTIVE CONTROL and REACTIVE CONTROL in triplicates on each plate with each run of specimens.
4. Blank values must have an absorbance of ≤ 0.100.
5. Non-Reactive Control values must have an absorbance of ≤ 0.100.
6. At least 2 of the 3 Reactive Control values must have absorbance ≥ 0.600. Any values outside of this range should not be used for calculation of the Reactive Control Mean (RCx).
7. If 2 Reactive Control values deviate more than 30% of the MEAN, the run is INVALID and should be repeated.
8. For invalid assays, refer to **TROUBLESHOOTING GUIDE**.

ASSAY CUT-OFF VALUE

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

The presence or absence of antibodies specific for HTLV-I/II is determined by relating the absorbance of the specimens to the CUT-OFF VALUE (COV) of the plate. The CUT-OFF VALUE is calculated as (0.25 + NRC Mean Absorbance):-

CUT-OFF VALUE (COV) = 0.25 + NRCx

CALCULATION OF RESULTS

1. Calculation of Non-Reactive Control Mean Absorbance (NRCx)

Example:	**Well No.**	**Absorbance**
	C1	0.020
	D1	0.021
	E1	0.022
	Total	0.063
	Mean	= 0.063 / 3 = 0.021

Individual Non-Reactive Control values should be ≤ 0.100 unit.

If one Non-Reactive Control value does not meet the above criteria, it must be excluded as aberrant. The Non-Reactive Control Mean (NRCx) should then be recalculated using the remaining individual Reactive Control values. All remaining individual Non-Reactive Control values must meet the above criteria or the assay is invalid and must be repeated.

2. Calculation of Reactive Control Mean Absorbance (RCx)

Example:	Well No.	Absorbance
	E1	1.221
	F1	1.144
	G1	1.298
	Total	3.663
	Mean	3.663 / 3 = 1.221

Individual Reactive Control values must be ≥ 0.600 unit.

If one Reactive Control value does not meet the above criteria, it must be excluded as aberrant. The Reactive Control Mean (RCx) should then be recalculated using the remaining individual Reactive Control values. All remaining individual Reactive Control values must meet the above criteria or the assay is invalid and must be repeated.

3. Calculation of CUT - OFF value(COV)

	CUT - OFF Value	= 0.250 + NRCx
Example:	NRCx	= 0.021
	CUT - OFF Value	= 0.250 + 0.021
		= 0.271

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the CUT - OFF value are considered **Non-Reactive** by the **MPD HTLV I/II ELISA 4.0**.
2. Specimens with absorbance values **greater than or equal to** the CUT - OFF value are considered **initially reactive** by the criteria of the **MPD HTLV I/II ELISA 4.0**and should be retested in duplicate before interpretation.
3. Specimens found Reactive on retesting may be interpreted to be r**epeatedly reactive** for antibodies to HTLV-I/II by the criteria of the **MPD HTLV I/II ELISA 4.0.**
4. Initially reactive specimens which are **Non-Reactive** on retesting are considered **negative** by the criteria of the **MPD HTLV I/II ELISA 4.0.**

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity

515 HTLV-I/II positive, 40 HTLV indeterminate and 11 HTLV-3/STLV-3 positive samples were studied in three sites, including one in-house, two in France. The results, summarised in Table 1, showed a detection rate of 100% for 515 confirmed HTLV-I/II positive samples, and nearly 72.7% (8/11) for HTLV-3/STLV-3 (simian counterpart of HTLV-3) positive samples.

Table 1: Detection Rate of Antibody to HTLV-I and HTLV-II in Various Groups of HTLV Samples

Sample Type	MPD HTLV-I/II ELISA 4.0			Confirmed Positive for Antibody to HTLV-I/II [a]
	No. of samples	Reactive	Negative	
HTLV-I	371	371	0	371
HTLV-II	134	134	0	134
HTLV-I/II co-infection	6	6	0	6
HTLV seropositive	4	4	0	4
HTLV Indeterminate	40	13	27	0
HTLV-3/STLV-3 [b]	11	8	3 [c]	NA

[a] All HTLV-I/II positive samples have been confirmed with an alternative ELISA and majority of the samples are further confirmed with MPD HTLV Blot 2.4 and/or PCR.
[b] All 11 samples are positive by PCR. Six of the samples are from Monkey (STLV-3) source. The other 5 samples accounted for serial bleed from 2 HTLV-3 infected individuals: Lobak18 (untypable profile on MPD HTLV Blot 2.4) and Pyl43 (tested as indeterminate with MPD HTLV Blot 2.4).
[c] These 3 samples are borderline negatives from Pyl43, whose HTLV-3 proviral load is very low as determined by PCR.

Specificity

A total of 5,306 samples comprising of random blood donor samples (n=5001), Clinical samples (n=205) and potentially interfering samples (n=100) were tested. The results, summarised in Table 2, showed a diagnostic specificity of 99.82% for the random blood donor, and 100% for clinical samples and potentially interfering samples.

Table 2: Diagnostic Specificity of MPD HTLV-I/II ELISA 4.0 in Various Groups of Samples

Sample Group	MPD HTLV I/II ELISA 4.0			Confirmed False Positive [a]
	No. of samples	Non-Reactive	Repeatedly Reactive	
Blood donor	5001	4990	11	9 (0.18%)
Hospitalized patient	205	204	1	0
Clinical pregnancy	50	50	0	0
HCV	10	10	0	0
HIV	20	17	3	0
H.pylori	10	10	0	0
Rheumatoid Factor	10	10	0	0
Total	5306	5291	15	9 (0.18%)

[a] All Repeatedly Reactive samples were further confirmed with MPD HTLV Blot 2.4 to rule out the true positive and indeterminate samples.

Reproducibility

The assay precision of the **MPD HTLV I/II ELISA 4.0** was evaluated in-house using three serum calibrators including a Reactive Control (RC), a HTLV-I positive sample and a HTLV-II positive sample.

Within-run: Three lots of ELISA components were assayed as 30 replicates per serum calibrator on 3 occasions. The coefficient of variation (CV) for the 3 calibrators in different run varied between 6.5% and 13.6% (Table 3).

Between-run: A total of 90 observations were recorded to assess between-run precision. These observations represent 3 runs using 3 lots of ELISA components with each serum calibrator. The coefficient of variation for the 3 calibrators varied between 8.2% and 15.7% (Table 3).

Total precision: Three lots of ELISA components were assayed as 4 replicates per serum calibrator on each occasion. This is repeated 36 times over a period of 40 days by 4 operators. The overall precision was assessed with 430 normalized data points (OD/COV) obtained with 3 serum calibrators. The coefficient of variation is 15.9%.

Table 3 Assay Reproducibility of MPD HTLV-I/II ELISA 4.0

Samples	Assay Components	No. of Replicates	Mean OD/COV	Within-run Precision (CV, %)	Between-run Precision (CV, %)
Reactive Control	#1	30	5.455	8.5	9.5
	#2	30	5.890	9.0	
	#3	30	6.053	8.0	
HTLV-I Positive	#1	30	5.380	6.5	8.2
	#2	30	5.355	10.9	
	#3	30	5.310	6.7	
HTLV-II Positive	#1	30	10.530	9.1	15.7

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Solution
1. Invalid assay due to out-of-specification absorbance value of Blank or NRC (>0.100)	• Incubation temperature out of range • Incorrect dispensing volume • Incubation time longer than specified • Incorrect dilution for Working Conjugate • Cross-contamination of controls • Control and Working Conjugate not well mixed • Component mixed-up from different kits • Insufficient washing	• *Check preparation of reagents, ,ensure reagents are well mixed before use* • *Check control for signs of contamination* • *Do not use components from different lot of kits* • *Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader*
2. Invalid assay due to out-of-specification absorbance value of RC (<0.600), or overall weak or completely no color development	• Incubation temperature out of range • Incorrect dispensing volume • Insufficient Incubation time • Incorrect dilution for Working Conjugate • Control and Working Conjugate not well mixed • Controls and test specimens not equilibrated to room temperature before assay • Deterioration in enzymatic activity of conjugate • Deterioration of microplate • Incorrect reading filter or Interference of optical pathway. • Component mixed-up from different kits • Excessive washing	• *Check preparation of reagents, ensure reagents are well mixed before use* • *Do not spin conjugate vial before use* • *Check control for signs of contamination* • *Check expiry date of kit and its components* • *Do not use components from different lot of kits* • *Equilibrate reagents to room temperature before use* • *Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader*
3. Valid assay but overall color development is too strong with too many initial reactive specimens	• Incubation temperature out of range • Incorrect dispensing volume • Incubation time longer than specified • Oxidization of substrate due to improper storage or use of dirty trough • Wrong preparation of 1x Wash Buffer • Deterioration of 1X Plate Wash • Insufficient or ineffective washing	• *Check preparation of reagents, ensure reagents are well mixed before use* • *Check control for signs of contamination* • *Check expiry date of kit and its components* • *Do not use components from different lot of kits* • *Equilibrate reagents to room temperature before use* • *Use clean containers and troughs* • *Check the quality of distilled or deionized water used for dilution* • *Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader* • *Check that 1X Plate Wash is within 2 weeks shelf-life after preparation*

LIMITATIONS OF THE PROCEDURE

Deviation from the recommended procedure may lead to aberrant results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no expressed 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 howsoever caused by the product in the use or in the application thereof.

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.

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