Revised 1 Dec. 2010 rm (Vers. 1.1) **RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

This HTLV-I/II ELISA Kit is to be used for detection of antibodies to T-lymphotropic viruses, type I and type II (HTLV-I/II) in human serum or plasma. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

This HTLV I/II ELISA kit is an enzyme-linked immunosorbent assay (ELISA) that can successfully detect HTLV-I/II antibodies in human serum or plasma using a highly antigenic polyprotein mixture derived from essential epitope sequences of HTLV-I core glycoproteins (p19), HTLV-I envelope (gp 46 and gp21) and HTLV-II envelope glycoprotein (p46).

PRINCIPLE OF THE ASSAY

This HTLV-I/II enzyme-linked immunosorbent assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with synthetic HTLV-I /II peptides. Samples or controls are added to the microtiter plate wells and incubated. HTLV I/II specific antibodies, if present, will bind the antigenic determinant of the HTLV I/II peptides and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove any HTLV-I/II antibodies and other components of the sample. A standardized preparation of horseradish peroxidase (HRP)-conjugated goat anti- human IgG antibodies is added to each well to "sandwich" the HTLV-I/II antibodies immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3',5,5' tetramthyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells containing HTLV-I/II specific antibodies and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. Samples with O.D values greater than or equal to the Cut-off Value are considered reactive by the criteria of this HTLV-I/II Antibody ELISA Kit.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

1. **MICROTITER PLATE** ***96 wells***
   Pre-coated with HTLV I/II synthetic peptides.

2. **CONJUGATE** ***15 mL***
   Horseradish peroxidase conjugated goat anti-human IgG antibody. *Ready-to-use.*

3. **BLANK CONTROL** ***1 mL***
   Buffered solution with animal protein and preservative.

4. **NON-REACTIVE CONTROL (NRC)** ***1 mL***
   Inactivated normal human serum diluted with Sample Diluent.

5. **HTLV-I REACTIVE CONTROL (RCI)** ***1 mL***
   Inactivated human serum with preservative.

6. **HTLV-II REACTIVE CONTROL (RCII)** ***1 mL***
   Inactivated human serum diluted with Sample Diluent.

7. **SAMPLE DILUENT** ***15 mL***
   Buffered solution with animal protein, preservative and color indicator.

8. **WASH BUFFER (20X)**) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

9. **SUBSTRATE A**) ***10 mL***
   Buffered solution with H_2O_2.

10. **SUBSTRATE B** ***10 mL***
    Buffered solution with TMB.

11. **STOP SOLUTION** ***14 mL***
    2N sulfuric acid solution (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 µL required for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator (37 ± 2°C)
8. Microtiter plate reader (450 nm ± 2 nm)
9. Automatic microtiter plate washer or squirt bottle
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Controls, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum, plasma and the Controls in the Kit should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All materials should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish colour prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING, AND STORAGE

a) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
b) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulates. *This HTLV-I/II Antibody ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin*.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum and plasma samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Blank Control, Non-Reactive Control, and Reactive Control:** NRC, **RC** and BC are supplied in a diluted form - no dilution is needed. See Assay Procedure.

2. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

3. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) before starting assay procedure (see Preparation of Reagents). *It is recommended that the table provided be used as a reference for adding Controls and samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
A1, B1	Blank Control (BC)	**G1, HI**	HTLV-II Reactive Control (RCII)
C1, D1	Non-Reactive Control (NRC)	**A2.....**	Samples
E1, F1,	HTLV-I Reactive Control (RCI)		

2. Remove the Microtiter Plate from the sealed pouch. If any coated strips are not to be used, return them to the pouch and seal tightly. Immediately place the remaining strips into storage (2-8°C).

3. Add 100 µL of Blank Control, Non-Reactive Control, HTLV-I Reactive Control and HTLV-II Reactive Control into the appropriate Microtiter Plate well. **Do not dilute the Controls**!

4. Prepare a 1:21 dilution of each sample by, first pipetting **100 µL** Sample Diluent into the appropriate Microtiter Plate well, then adding **5µL** of sample. **The color of the sample diluent will change to blue after adding the sample.** Mix well. Cover and incubate the Microtiter Plate for **60 minutes at 37°C ± 2°C.**
5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of five washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **five times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes*.

6. Add **100 µL** Conjugate to each well. Cover and incubate plate for **30 minutes at 37°C ± 2°C**.

7. Prepare Substrate Solution (see Preparation of Reagents section) no more than 15 minutes before end of second incubation.

8. Repeat wash procedure as described in Step 5.

9. Add **100 µL** Substrate Solution to each well. Cover and incubate Microtiter Plate for **15 minutes at 37°C ± 2°C**.

10. Add **100 µL** Stop Solution to each well. Mix well..

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

RESULTS

1. All O.D. values for Controls and samples are subtracted by the O.D. value of the Blank Control before result interpretation.

2. If more than one plate is being assayed at the same time, each plate must be calculated and interpreted separately.

3. The presence or absence of antibody to HTLV-I/II is determined by relating the O.D. of the samples to the CUT-OFF value.

CALCULATION OF RESULTS

1. **Calculation of Blank Control Mean O.D. (BC)**

Example:	***Well No.***	***O.D***		
	A1	0.039		
	B1	0.058		
	Total	0.097		
	Mean	0.097/2	=	0.049 (BC)

2. **Calculation of Non-Reactive Control Mean O.D. (NRC)**

Example:	***Well No.***	***O.D***	***BC subtracted***	
	C1	0.042	-0.007	
	D1	0.067	0.018	
	Total		0.011	
	Mean		0.011/2	= 0.006 (NRC)

3. **Calculation of HTLV-I Reactive Control Mean O.D. (RCI)**

Example:	***Well No.***	***O.D***	***$\overline{BC}$ subtracted***	
	E1	1.651	1.602	
	F1	1.732	1.683	
	Total		3.285	
	Mean		3.285/2	= 1.643 (RCI)

4. **Calculation of HTLV-II Reactive Control Mean O.D. (RCII)**

Example:	***Well No.***	***O.D***	***BC subtracted***	
	G1	1.067	1.018	
	H1	0.930	0.881	
	Total		1.899	
	Mean		1.899/2	= 0.950 (RCII)

5. **Calculation of $\overline{\text{RCI}}$ - $\overline{\text{NRC}}$**

Example:

$\overline{\text{RCI}}$ = 1.643

$\overline{\text{NRC}}$ = 0.006

$\overline{\text{RCI}} - \overline{\text{NRC}}$ = 1.643-0.006

= 1.637

6. **Calculation of CUT-OFF Value.** (If Non-Reactive Control O.D. value is lower than Blank Control, the cut-off value will be zero plus 0.22.)

CUT-OFF value = 0.22 + $\overline{\text{NRC}}$

Example:

$\overline{\text{NRC}}$ = 0.006

CUT-OFF value = 0.22 + 0.006

= 0.226