

For Research Use Only Not for Diagnostic Use



HTLV SCREENING

HTLV 1+2

Catalog #: WH9196

**HUMAN T-CELL LYMPHOTROPIC VIRUSES
ANTIBODY ELISA KIT**
**ONE Step Incubation, Double Antigen Sandwich
Principle**

INSTRUCTIONS FOR USE

This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antibodies to Human T-Cell Lymphotropic Virus types 1 and/or 2 (HTLV-1/2) in human serum or plasma. It is intended for screening of blood donors and as an aid for the research of clinical conditions related to infection with HTLV-1 and/or HTLV-2.

SUMMARY

The human T-cell lymphotropic viruses (HTLV) is a member of the family of Retroviridae, consisting of enveloped double stranded RNA viruses and genetically not related to HIV1&2; however, they have similar routes of transmission and can have extremely long period of latency prior to manifestation of disease. HTLV type 1 was reported in 1980 as the first retrovirus shown to be pathogenic to humans. The virus preferentially infects CD4+ lymphocytes while the infections of CD8+ T lymphocytes are rare. In contrast to HTLV 1, HTLV type 2 can infect all type of lymphocytes as well as the macrophages. HTLV 1&2 is transmitted transplacentally, parenterally, by sexual contacts and by infected blood. The diseases associated with HTLV infection are usually classified as malignant or nonmalignant clinical presentations. HTLV 1 is endemic in southern Japan, the Caribbean and the US and many other scattered population through the world. HTLV 2 is endemic in some North American Indian tribes but is detected mostly in intravenous drug users and their sexual partners.

PRINCIPLE OF THE ASSAY

This kit is one step incubation, antigen "sandwich" enzyme immunoassay (ELISA) method, which uses polystyrene microwell strips pre-coated with recombinant HTLV antigens expressed in E.coli. Patient's serum/plasma sample is incubated in the microwells together with second recombinant HTLV antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-HTLV in sample, the pre-coated and HRP-conjugated

antigens will be bound to the two variable domains of the antibody and during incubation, the specific antigen-antibody immunocomplex is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugate, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen(HRP) "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HTLV remain colorless.

COMPONENTS

- **MICROWELL PLATE** 1 plate
Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. 12×8/8×12-well strips per plate. Each well contains recombinant HTLV 1/2 antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.
- **NEGATIVE CONTROL** 1 vial
Yellowish liquid filled in a vial with green screw cap. 0.3 ml per vial. Protein-stabilized buffer tested non-reactive for HTLV 1/2. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8 °C.
- **POSITIVE CONTROL** 1 vial
Red-colored color liquid filled in a vial with red screw cap. 0.3ml per vial. Antibodies to HTLV 1/2 diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8 °C.
- **HRP-CONJUGATE REAGENT** 1 vial
Red-colored liquid filled in a white vial with red screw cap. 6.5ml per vial. Horseradish peroxidase-conjugated HTLV 1/2 antigens. Ready to use as supplied. Once open, stable for one month at 2-8 °C.
- **STOCK WASH BUFFER** 1 bottle
Colorless liquid filled in a clear bottle with white screw cap. 50ml per bottle. pH 7.4 10 × PBS (Containing Tween-20 as a detergent). **DILUTE BEFORE USE** -The concentration must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks at 2-8 °C.
- **CHROMOGEN SOLUTION A** 1 vial
Colorless liquid filled in a white vial with green screw cap. 7ml per vial. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8 °C.
- **CHROMOGEN SOLUTION B** 1 vial
Colorless liquid filled in a black vial with black screw cap. 7ml per vial
TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8 °C.
- **STOP SOLUTION** 1 vial
Colorless liquid filled in a white vial with yellow screw cap. 7ml per vial.
Diluted sulfuric acid solution (2.0M H₂SO₄).
- **PLASTIC SEALABLE BAG** 1 unit
For enclosing the strips not in use.
- **CARDBOARD PLATE COVER** 2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

● **PACKAGE INSERTS**

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ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5 °C.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22µ filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolyzed samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8 °C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the

content of the wells but allow the plate washer to aspirate it automatically.

4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50 ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C; **do not freeze**. To assure maximum performance of this anti-HTLV 1+2 ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY**

FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.

7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H₂SO₄) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

ASSAY PROCEDURE

- Step 1 Reagents preparation:** Allow the reagents to reach room temperature (**18-30°C**) for at least **15-30minutes**. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.
- Step 2 Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls (e.g. **B1, C1, D1**), two Positive controls (e.g. **E1, F1**), and one Blank (e.g. **A1**, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required.
- Step 3 Adding HRP-Conjugate:** Add **50µl** HRP-Conjugate to each well except the Blank.
- Step 4 Adding Sample:** Add **50µl** of Positive control, Negative control, and specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.**
- Step 5 Incubating:** Mix gently. Cover the plate with the plate cover and incubate for **60minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.
- Step6 Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it as to remove any reminders.
- Step7 Coloring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, cover the plate with plate cover and mix by tapping the plate gently. Incubate the plate at **37°C for 30minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in positive control and HTLV 1/2 Positive sample wells.
- Step8 Stopping Reaction:** Remove and discard the plate cover. Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow color develops in positive control and HTLV 1/2 positive sample wells.
- Step9 Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **5 minutes** after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of the Cut-off value (C.O.) = *NC + 0.18

*NC = the mean absorbance value for three negative controls

Example:

1. Calculation of NC:

Well No	B1	C1	D1
Negative Controls OD value	0.028	0.030	0.032

NC=0.030

2. Calculation of Cut-off (C.O.) = 0.030 + 0.18 = 0.210

If one of the Negative Control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be equal to or less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1) : Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HTLV 1/2 antibodies have been detected with this HTLV 1/2 ELISA kit, therefore the patient is probably not infected with HTLV 1/2.

Positive Results (S/C.O. ≥1) : Samples giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HTLV 1/2 antibodies have probably been detected using this HTLV 1/2 ELISA kit.

Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HTLV 1/2 and

therefore there are serological indications for infection with HTLV 1/2.

Borderline (S/C.O. =0.9-1.1) : Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples could be considered positive for antibodies to HTLV 1/2.

Follow up and supplementary testing of any positive samples with other analytical system (e.g. WB, PCR) is required before establishing of the final diagnosis.

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological of ELISA systems. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the "sandwich model" minimizes the unspecific reactions due to interference with unknown matters in sample. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. Common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. The assay cannot distinguish between infections with HTLV-1 and HTLV-2.
8. This is a qualitative assay and the results cannot be used to measure antibodies concentrations.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.

VALIDITY

Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels

REFERENCES:

1. Engval E. et al.. J Immunochemistry, 1971; 8: 871-874.
2. Engval E. et al.. J Immunol. 1971; 109:129-135.
3. Takeyuki Kohno et al.. J Clin Lab Anal. 1992; 6:105-112
4. Brodine, S. K., E. M. Kaime, C. Roberts, R. P. Turnicky, and R. B. Lal. 1993. Simultaneous confirmation and differentiation of human T-lymphotropic virus types I and II infection by modified Western blot containing recombinant envelope glycoproteins. Transfusion 33:925-929
5. Centers for Disease Control and Prevention and the USPHS Working Group. 1993. Guidelines for counseling persons infected with human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II). Ann. Intern. Med. 118:448-454.
6. Public Health Service Working Group. 1988. Licensure of screening test for antibody to human T-lymphotropic virus type I. Morb. Mortal. Wkly Rep. 37:736-747.
7. Roberts, B. D., S. K. Foung, J. J. Lipka, J. E. Kaplan, K. G. Hadlock, G. P. Reyes, L. Chan, W. Heneine, and R. F. Khabbaz. 1993. Evaluation of an immunoblot assay for serological confirmation and differentiation of human T-cell lymphotropic virus types I and II. J. Clin. Microbiol. 31:260-264.

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