



HTLV I+II

ELISA recombinante v.4.0

Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to HTLV I & II viruses

SUMMARY

The lymphotropic viruses from human T cells, HTLV type that belong to the family of retroviruses are divided into two types: HTLV-I and HTLV-II. They are associated with malignant T cells diseases and degenerative neurological processes.

The HTLV-I is the etiological cause of two types of pathologies: adult T-cell leukemia (ATL) and various neurological disorders involving demyelination including myelopathy associated to HTLV-I (HAM) and tropical spastic paraparesis (TSP). Also specific HTLV-I antibodies are detected in certain cases of dermatitis, uveitis, polymyositis, and arthritis.

HTLV-II has been associated with neurological syndromes similar to HAM.

Both viruses are transmitted by sexual contact, exposure to contaminated blood transfusion, or transmission from an infected mother to fetus during the prenatal period or by breast milk.

The **HTLV I+II ELISA recombinante v.4.0** kit is designed to detect HTLV-I and HTLV-II antibodies. It may be used in the diagnosis of infection and donor units control in blood banks.

PRINCIPLE

The microtitration plate wells are coated with the recombinant antigens to HTLV I&II viruses. The diluted sample is incubated in the wells. If antibodies against one of the viruses are present in the sample, they bind to the antigen well. The unbound fraction is removed by washing. In the next step the conjugate is added, consisting of HTLV I and HTLV II antibodies conjugated to peroxidase. It binds to the antigen-antibody complexes previously formed. The unbound conjugate is removed by washing. Then, a solution containing tetramethylbenzidine and hydrogen peroxide is added. Reactive samples develop a light-blue color that changes to yellow when the reaction is stopped with sulfuric acid (Stopper).

PROVIDED REAGENTS

Coated microtitration plate: microtitration plate with cut-out strips and 96 wells coated with HTLV I&II recombinant antigens.

Sample Diluent: saline buffer with surfactant. Violet color.

Conjugate: antigens anti-HTLV I and HTLV II conjugated to peroxidase. Red color.

TMB: 36 mM tetramethylbenzidine solution in 100% dimethylsulphoxide (DMSO), (100x).

TMB diluent: 40 mM citrate buffer and 1.27 mM hydrogen peroxide, pH 4.3.

Stopper: 2 N sulfuric acid.

Concentrated Wash Buffer: saline buffer with surfactant (25x). Green color.

Positive Control: inactivated human serum containing antibodies to HTLV I and/or HTLV II. Orange color.

Negative Control: inactivated non-reactive human serum. Yellow color.

NON-PROVIDED REAGENTS

Distilled or deionized water.

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring stated volumes
- Disposable tips
- Volumetric material to prepare stated dilutions
- 37°C incubator
- Absorbent paper
- Disposable gloves
- Timer or stopwatch
- Sodium hypochlorite
- Microtitration plate washing system (manual or automatic)
- Spectrophotometer for microtitration plate reading

WARNINGS

- The reagents are for "in vitro" diagnostic use.
- All patient samples should be handled as capable of transmitting infection. The controls are inactivated. However, they should be used as infective material.
- The control sera have been tested for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and found to be non-reactive. Due to the high level of HIV co-infection, certain reactive controls for HTLV I+II can also be reactive for HIV. Thus, they should be handled as potentially infectious material.
- All materials used to perform the test must be treated to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for one hour at 121°C. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- Avoid contact of reagents with hypochlorite fumes from biohazards disposal containers or other sources, since hypochlorite affects the reaction.
- Avoid liquid spilling and aerosol formation.
- Do not use the reagents after the expiration date.
- Do not exchange reagents from different lots and do not modify assay procedures.
- Do not use reagents from other origin.
- Avoid touching the walls of the wells with the tips.
- Avoid using metal objects that may be in contact with the reagents.

- The microtitration plates should be placed in incubator. Do not use water bath. Do not open the incubator during this process.
- Sulfuric acid (Stopper) is corrosive. H315+H320: Causes skin and eye irritation. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/eye protection/face protection.
- Do not pipette by mouth. Use disposable gloves and eye protection during test sample and reagent handling.
- TMB is sensitive to light. Keep the bottle capped when unused.
- All reagents and samples should be discarded according to current regulations.

REAGENTS PREPARATION

All the material used for reagent preparation should be clean and free from detergent and hypochlorite.

Wash Buffer: constituents of the concentrated reagent may precipitate at low temperature. In such case, bring the solution to 37°C until complete dissolution. To obtain a ready-to-use wash buffer (1x) dilute 1 part Concentrated Wash Buffer (25x) with 24 parts distilled or deionized water. Example: 20 ml with 480 ml for one microtitration plate.

Substrate: in a labeled tube, dilute the concentrated TMB (100x) in TMB Diluent. E.g. 200 µl in 20 ml for a microtitration plate. Concentrated TMB is dissolved in DMSO. Since the melting temperature of the DMSO is 18 °C the TMB must reach room temperature (20-25 °C) and be completely homogenized before use.

Coated microtitration plate, Sample Diluent, Conjugate Diluent, Substrate, Stopper, Negative and Positive Controls: ready to use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable in refrigerator (2-10 °C) until expiration date stated on the box. Do not freeze.

Concentrated Wash Buffer and Stopper: store at room temperature (2-25 °C).

Wash Buffer (1x): store in sealed container. Stable for 3 months at room temperature (2-25 °C).

Coated microtitration plate: do not open the pouch until performing the test and until it has reached room temperature. Otherwise, the well strip surface could get moistened. Unused well strips should be kept in the pouch with the desiccant, sealed with adhesive tape and stored at 2-10 °C. Test strips stored in this manner are stable for 4 months if they do not exceed the date printed on the pouch label.

Substrate: once prepared it should be used within 3 hours keeping it protected from light at room temperature (2-25 °C). Discard if coloration is observed.

SAMPLE

Serum or plasma

a) Sample collection: obtain in the usual way.

b) Additives: not required for serum. Use plasma collected

with heparin, citrate, fluoride or EDTA as anticoagulants.

c) Known interfering substances: no interference has been observed with bilirubin up to 30 mg/dl, ascorbic acid up to 50 mg/dl, triglycerides up to 750 mg/dl, hemoglobin up to 300 mg/dl. Samples containing particles should be clarified by centrifugation.

d) Stability and storage instructions: sample should be stored refrigerated (2-10°C) up to 3 days. If extended storage periods are required, samples should be frozen at -20°C (or less). Samples should not be repeatedly frozen and thawed. This may lead to erroneous results. In case of using frozen samples, they should be homogenized and centrifuged before use.

Heat inactivation may affect the result.

Do not use samples with microbial contamination.

If samples are to be transported, they should be packaged according to local regulations for biohazard material shipment.

TEST PROCEDURE

1- Bring the reagents and samples to room temperature before starting the test.

2- Prepare the necessary volume of Wash Buffer (1x).

3- Place the required wells in the strip holder for the number of determinations to be used, including 2 wells for Positive Control (PC) and 3 for Negative Control (NC).

4- Dispense the Sample Diluent, then the sample (S) and the controls according to the following scheme:

	M	PC	NC
Sample Diluent	50 µl	50 µl	50 µl
Positive Control	-	50 µl	-
Negative Control	-	-	50 µl
Sample	50 µl	-	-

Homogenize 2-3 times by loading and unloading the micropipette or shaking the plate for 10 seconds. When adding the sample, the Sample Diluent will change color (see table).

Sample type	Without sample	Serum or plasma	Positive Control	Negative Control
Color	Violet	Light blue	Dark orange	Green

Control or sample dosing to the wells may be visually or spectrophotometrically verified (at 610/650 nm).

Warning: turbid, icteric or hemolyzed samples may change the final color without affecting the results. Color change may depend on the added sample volume and its composition. A less intense color change may be due to a lower dispensed sample volume, the sample not being in the appropriate conditions, or a low protein level.

5- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 30 ± 2 minutes at 37 ± 1°C.

6- The liquid from each well must be thoroughly removed after incubation. Wash 5 times according to washing instructions (see WASHING PROCEDURE).

7- Add the Conjugate:

Conjugate	100 ul	100 ul	100 ul
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To avoid evaporation cover the microplate with adhesive tape.

8- Incubate for 30 ± 2 minutes at $37 \pm 1^\circ\text{C}$.

9- Wash 5 times according to the washing instructions.
 10- Prepare de Substrate (See REAGENT PREPARATION). Dispense the Substrate. Transfer only the required Substrate volume to a clean recipient. Do not transfer the remaining Substrate back to the original bottle. Avoid reagent contact with oxidizing agents.

Substrate	100 ul	100 ul	100 ul
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11- Incubate for 30 ± 2 minutes at room temperature ($18\text{-}25^\circ\text{C}$), protecting from light.

12- Add the Stopper:

Stopper	100 ul	100 ul	100 ul
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13- Read absorbance in spectrophotometer monochromatically at 450 nm or bichromatically at 450/620-650 nm.

Note: bichromatic reading is recommended. In case the reading is monochromatic, perform a reagent blank that will have to be subtracted from all sample values.

STABILITY OF THE FINAL REACTION

Reaction color is stable for 10 minutes. Thus, results should be read within that period.

WASHING PROCEDURE

Remove the liquid from the wells by aspiration or inversion. The wells are washed with 350 ul diluted Wash Buffer. When filling the wells make sure not to spill. The wash solution should be in contact with the wells for 30 to 60 seconds. Make sure no residual liquid remains after the final washing step. Perform double aspiration to remove excess buffer. If after such procedure, it still persists, invert the plate onto absorbent paper and tap it several times. Otherwise, erroneous results may be obtained.

Note: the washing procedure is crucial for the test result. If excess wash buffer remains in the wells, or if the wells are not completely filled, erroneous results may be obtained. Do not let the wells air dry during the procedure. Automatic washers should be rinsed with distilled or deionized water at the end of the day to avoid obstructions due to the presence of salt in the wash buffer.

SUMMARY OF THE PROCEDURE

STAGE	PROCEDURE	WARNINGS/OBSERVATIONS
Dilution	Prepare Wash solution (1x)	Dissolve salt crystals
Sample Diluent	Add 50 ul Sample Diluent in each well	
Samples	Add 50 ul S, PC and NC	Color change is observed when adding the sample and controls
Incubation	Cover the wells and incubate for 30 ± 2 minutes at $37 \pm 1^\circ\text{C}$	In incubator
Washing Step	Wash each well with 350 ul diluted Wash Buffer (5 times)	Time of contact of the Wash solution from 30 to 60 seconds. Completely remove the residual liquid from the wells
Conjugate	Add 100 ul Conjugate	
Incubation	Cover the wells and incubate for 30 ± 2 minutes at $37 \pm 1^\circ\text{C}$	In incubator
Washing Step	The same as above	
Dilution	Prepare Substrate (1x)	During incubation with the conjugate, dilute TMB100x with TMB diluent
Substrate	Add 100 ul Substrate	Discard the remaining reagent. Avoid contact with oxidizing agents. Do not expose to light.
Incubation	30 ± 2 minutes at $18\text{-}25^\circ\text{C}$	Maintain the wells protected from light
Stop	Add 100 ul Stopper	
Reading	Read in spectrophotometer	Read within 10 minutes

ASSAY VALIDATION CRITERIA

The assay is considered valid if the following conditions are simultaneously met:

1- The mean absorbance of the Negative Controls should be less than or equal to 0.150

Example:

Reading 1 = 0.060 Reading 2 = 0.055. Reading 3 = 0.070
Mean = $(0.060 + 0.055 + 0.070)/3 = 0.055$

2- Remove any Negative Control with absorbance greater than 0.150

3- If any Negative Control has been removed recalculate the Negative Control mean. Any assay is valid when at least two of the Negative Controls are accepted.

4- The absorbance mean of the Positive Controls should be greater than 0.900.

Example:

Reading 1 = 1.358. Reading 2 = 1.214
Mean = $(1.358 + 1.214)/2 = 1.286$

5- The absorbance mean difference of the Positive and Negative Controls should be greater or equal to 0.750.

In case one of the above conditions is not met, repeat the assay.

Remember that the obtained readings will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

The presence or absence of antibodies anti-HTLV I and/or II is determined associating the sample absorbance with the Cut-off value.

Cut-off = NC + 0.200

NC: absorbance mean of the Negative Control

Example: $0.035 + 0.200 = 0.235$

Non-reactive samples: those with absorbance lower than the Cut-off value.

Reactive samples: those with absorbance greater or equal to the Cut-off value.

All samples initially reactive should be repeated by duplicate. If one or both replicates are reactive, it should be considered reactive.

A sample initially reactive may be non-reactive in both replicates. This may be due to:

- Cross contamination of a non-reactive well with a reactive sample.
- Sample contamination during dispensation, lack of precision in sample, conjugate and/or Substrate dispensation into the well.
- Tip re-utilization.
- Well contamination with hypochlorite or other oxidizing agents.

In certain cases a non-reactive sample may produce a

falsely reactive reaction, both in the initial analysis as in its replicates. Some probable causes of this effect may be:

- Sample contamination during collection, processing or storage.
- Presence of interfering substances, such as autoantibodies, drugs, etc.
- Ineffective dispensation and/or aspiration of the wash solution (obstructed system).

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

Do not use pooled samples.

Do not use other body fluids such as saliva, cerebrospinal fluid or urine.

Repeatedly reactive samples should be tested using supplemental or confirmatory techniques, according to local regulations.

SPECIFIC PERFORMANCE FEATURES

a) Sensitivity

Sensitivity in performance panels

In a study performed on different international commercial panels, the following results were obtained:

PRP205 (M) (Anti-HTLV I/II Mixed Titer Performance Panel Modified, BBI, USA) 18 out of 18 reactive samples were detected.

PRP206 (Anti-HTLV I/II Mixed Titer Performance Panel, Panel, BBI, USA) 14 out of 14 reactive samples were detected.

PRP207 (Anti-HTLV I/II Mixed Titer Performance Panel, BBI, USA) 14 out of 14 reactive samples were detected.

QRP 712 (Anti-HTLV I/II Qualification Panel, SERACARE. USA) 5 out of 5 reactive samples were detected.

QRP 751 (Anti-HTLV I/II Qualification Panel, SERACARE. USA) 4 out of 4 reactive samples were detected.

QRP 761 (Anti-HTLV I/II Qualification Panel, SERACARE. USA) 5 out of 5 reactive samples were detected.

Clinical sensitivity in anti-HTLV I and/or II reactive sample panels

In a study performed on 47 samples with HTLV I and/or II infection, confirmed by different methods, all of the samples were found reactive with the HTLV I+II ELISA recombinante v.4.0 kit.

b) Specificity

In a study performed on 921 sera samples from different health centers of Rosario city, the obtained specificity was 99.67%.

In a further study performed on 1048 plasma samples from different health centers of Rosario city, the obtained specificity was 100%.

A possible cross-reactivity was evaluated. Assaying 184 samples from individuals with different clinical conditions that may be the cause of unspecific reactions for the **HTLV I+II ELISA recombinante v.4.0** test. This group included samples:

- With antibodies to HAV, HBV, EBV, CMV, HSV, VZV, HIV, Chagas and other viruses.

- With different autoantibodies (AGA, AMA, ATA, FAN, Rheumatoid factor and others).
 - With antibodies to *Treponema pallidum*, *Mycoplasma pneumoniae*, *Toxoplasma gondii*, *Toxocara canis*, *Trypanosoma cruzi* and other microorganisms.
 - From hemodialyzed patients and pregnant women.
- The obtained specificity for this population was 100%.

- of HTLV-2 in blood donors from Arequipa. Perú. Rev. Inst. Med. trop. S. Paulo 51(1):25-29. 2009.
- Anna Abrams. Yoshimi Akahata and Steven Jacobson. The Prevalence and Significance of HTLV-I/II Seroindefinite Western Blot Patterns. Viruses 2011. 3. 1320-1331.

c) Precision

The test precision was evaluated following EP15-A protocol recommended by the NCCLS. The assays were performed with samples having different HTLV I & II reactivity levels and with controls. Two daily assays were performed testing each sample by duplicate during 5 days.

	Mean	Intra-assay		Total	
	PI (OD/CO)	S	CV	S	CV
Sample 1	1.25	0.071	5.72%	0.094	7.57%
Sample 2	1.54	0.083	5.42%	0.090	5.86%
Sample 3	3.38	0.149	4.41%	0.177	5.25%
Sample 4	5.21	0.177	3.39%	0.239	4.58%
Sample 5	2.80	0.076	2.72%	0.127	4.53%
Sample 6	1.39	0.056	4.03%	0.092	6.62%
Positive Control	9.32	0.352	3.77%	0.460	4.94%
Negative Control	0.10	0.010	9.64%	0.017	17.05%

n= 20

WIENER LAB. PROVIDES

Kit for 96 determinations (Cat. Nr. 1671096)

REFERENCES

- Feuer G. Green PL. Comparative biology of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2. Oncogene 24(39):5996-6004 (2005).
- Roucoux DF. Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus II. AIDS Rev 3:144-54. (2004).
- Poiesz BJ. Poiesz MJ. Choi D. The human T-cell lymphoma/leukemia viruses. Cancer Invest 2 :253-77. (2003).
- Gotuzzo E. Arango C, de Queiroz-Campos A. Istúriz RE. Human T-cell lymphotropic virus-I in Latin America. Infect Dis Clin North Am. 14(1):21 1-39, x-xi (2000).
- User Demonstration of Performance for Precision and Accuracy – Approved Guideline EP15-A (2001).
- Clinical Evaluation of Immunoassays. Approved Guideline I/LA21-A (2002) National Committee for Clinical Laboratory Standards.
- Interference testing in Clinical Chemistry. Approved Guideline EP7-A (2002) National Committee for Clinical Laboratory Standards.
- Richard Malan. Carolina A. Berini. Seroprevalencia de HTLV-1/2 en donantes de sangre de la provincia de Misiones. Medicina (Buenos Aires) 2009; 69: 71-74.
- Nadia C. Santos Quispe. Edwin Bengoa Fera. Elizabeth de los Santos-Fortuna and Adele Caterino-de-Araujo. Confirming the presence of htlv-1 infection and the absence

SYMBOLS EXPLANATION

Policubeta	Sensib.	Diluyente	Muestra
Coated microtitration plate		Sample Diluent	
Conjugado	TMB	Diluy.	
Conjugate	TMB Diluent		
TMB	Buf. Lavado	Conc.	
TMB	Concentrated Wash Buffer		
Control +	Control -		
Positive Control	Negative Control		

Stopper

Stopper

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC **REP** Authorized representative in the European Community

IVD "In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution

Cont. Contents

LOT Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL - Negative Control

REF Catalog number

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