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Ref: 291/MKT/E/30

SUBJECT **Bioelisa HTLV I+II 5.0**
Product improvement

Dear customer,

With the aim of continuous development of its products, biokit presents a new generation of the former bioelisa HTLV- I+II (r).

The solid phase and protocol of this assay have been modified to increase user friendliness and performance in terms of sensitivity and specificity.

As a totally new and improved product, biokit has given it a new name and new code:

bioelisa HTLV- I+II 5.0 192 Tests, Cod. 3000-1165

Bioelisa HTLV- I+II 5.0 includes several features that make it an outstanding product:

- Third generation: Labelled antigen as conjugate
- Sample Addition monitoring
- Fast assay: A single incubation for sample and conjugate
- Improved Specificity: No plate background.
- Better sensitivity: Total antibody detection

Please find enclosed a complete product information sheet that we hope will help you to launch this assay. Good sales!



bioelisa HTLV-I+II 5.0

Product improvements:

Recombinant antigens

bioelisa HTLV-I+II 5.0 uses a combination of three epitopes collected in a single tri-fusion recombinant antigen. The assay assures the detection of both HTLV-I and HTLV-II by two type specific epitopes derived from the ENV antigens: gp46^I from HTLV-I and gp46^{II} from HTLV-II. A third common ENV antigen, gp21, enhances the sensitivity and specificity for both HTLV-I and HTLV-II.

gp21 is the best antigen for detecting HTLV as its correspondent antibody is the first to appear in the seroconversion of a patient. The gp21 recombinant is also a very specific antigen that does not cross-react with any other viral antigen.

Third generation assay

bioelisa HTLV-I+II 5.0 now uses a peroxidase labelled antigen as a conjugate instead of the former anti-human IgG. It is a real sandwich assay in which all kinds of anti-HTLV class antibodies bind simultaneously to the plate coating and conjugate. Specificity and microplate background increases dramatically in this kind of assay format.

Protocol: Single incubation for sample and conjugate

bioelisa HTLV-I+II 5.0 now uses a single incubation for sample and conjugate. Working conjugate is first dispensed into the microwells. The samples are then dispensed directly into the corresponding microwells. A change in the green tonality helps monitor sample addition. This change can also be read spectrophotometrically at 450 nm but definition of the OD limit must be validated customer by customer.

Sample and conjugate mixture is incubated for only one hour at 37 °C.

After 6 wash cycles, ready-to-use TMB substrate is added and incubated for 30 minutes at 37 °C.

Test name	bioelisa HTLV-I+II 4.0	bioelisa HTLV-I+II 5.0
Code number	3000-1156	3000-1165
Method	EIA/HRP/TMB	EIA/HRP/TMB
Format	2 - step indirect method	1 - step direct sandwich
Solid phase	12x8 Microtiter wells	12x8 Microtiter wells
Tests per kit	192	192
Coating Antigens	Recombinant antigens	Single tri-fusion Recombinant Antigen
HTLV-I, HTLV-II	gp46 ^I and gp46 ^{II}	gp46 ^I and gp46 ^{II}
Common HTLV	gp21	gp21
Conjugate	Goat anti-human IgG labelled with HRP	HTLV tri-fusion antigen labelled with HRP
Substrate	TMB	TMB
Specimen dilution	1/10 In plate dilution 20 µl sample 200 µl diluent	1/2 In plate dilution 50 µl Working conjugate 50 µl Direct sample
Sample incubation	60 min / 37°C	60 min / 37°C
Conjugate incubation	30 min / 37°C	No Applicable
Substrate incubation	15 min / 37°C	15 min / 37°C
Cut-off	Mean Neg. + 0.450	Mean Neg. + 0.250
Sensitivity	99.7%	100%
Specificity	99.80% Blood donors	99.82% Blood donors

Performance:

Sensitivity:

515 HTLV-I/II positive, 40 HTLV indeterminate and 11 HTLV-3/STLV-3 positive samples were studied at three sites, one in-house and two in France. The results, summarised in table 1, show 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.

Sample Type	bioelisa HTLV-I+II 5.0			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

BBI HTLV mixed titer PRP 205 (M)

				bioblot HTLV				
Member	HTLV type	bioelisa HTLV-I+II 5.0 OD/COV	Ortho HTLV OD/COV	gp21	p19	p24	II	I
BBI-205-1	1	5.32	4.9	+	+	+		+
BBI-205-2	2	11.69	2.1	+		+	+	
BBI-205-3	2	39.40	4.1	+		+	+	
BBI-205-4	2	39.41	4.9	+	+	+	+	
BBI-205-5	2	9.91	4.8	+	+	+	+	
BBI-205-6	neg	0.11	0.3	No bands				
BBI-205-7	1	13.8	4.9	+	+	+		+
BBI-205-8	2	4.00	2.5	+	+	+	+	
BBI-205-9	2	1.24 (dil 1/20)	3.3	+		+	+	
BBI-205-10	2	NA	3.6	+		+	+	
BBI-205-11	1	39.4	4.7	+	+	+		+
BBI-205-12	1	8.39	4.7	+	+	+		+
BBI-205-13	2	5.60	3.1	+	+	+	+	
BBI-205-14	1	1.84	4.6	+	+	+		+
				bioblot HTLV				
Member	HTLV type	bioelisa HTLV-I+II OD/COV	Ortho HTLV OD/COV	gp21	p19	p24	II	I
BBI-205-15	1	NA	4.9	+	+			+
BBI-205-16	2	11.32	3.9	+	+	+	+	
BBI-205-17	1	4.65	4.9	+	+	+		+
BBI-205-18	2	39.40	4.2	+		+	+	
BBI-205-19	1	NA	4.3	+	+			+
BBI-205-20	1	39.40	4.9	+	+	+		+
BBI-205-21	2	4.16	3.9	+	+	+	+	
BBI-205-22	2	3.93	1.9	+	+	+	+	
BBI-205-23	2	NA	1.8	+		+	+	
BBI-205-24	neg	0.11	0.2	No bands				
BBI-205-25	1	39.4	4.9	+	+	+		+

Specificity

A total of 5,306 samples comprised 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, show a diagnostic specificity of 99.82% (4990/4999) for the random blood donor, and 100% for clinical samples and potentially interfering samples.

Sample Group	bioelisa HTLV-I+II 5.0			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%)

Enclosed material:

- ✓ CE Mark
- ✓ Package insert
- ✓ Safety Data Sheet

CE MARK

DECLARACIÓN CE DE CONFORMIDAD
CE DECLARATION OF CONFORMITY



Don José Luis Zarroca Alberola, en calidad de Director General de Biokit S.A.
Mr. José Luis Zarroca Alberola, as General Manager of Biokit S.A.

DECLARA / DECLARES:

Que el producto / *That the product:* **bioelisa HTLV-I+II 5.0**

Código / *Code:* **3000-1165**

cumple con las exigencias mencionadas en el Anexo I y los Requisitos
Esenciales de la Directiva 98/79/CE.

*meets the provisions referred to in Annex I and Essential Requirements of the
Directive 98/79/CE.*



José Luis Zarroca Alberola

Lliçà d'Amunt, a 10 de Junio de 2009

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UL International (UK) Ltd

An affiliate of Underwriters Laboratories Inc.

EC Design – Examination Certificate

(Annex IV section 4 of the Directive 98/79/EC on In Vitro Diagnostic Medical Devices)

Manufacturer

Biokit SA
Can Malé
08186 Lliçà d'Amunt
Barcelona
Spain

Authorised Representative

Not Applicable

Model Type: Bioelisa HTLV-I+II 5.0 product code 3000-1165 (192T)

We hereby declare that a design examination has been carried out on the device(s) listed following the requirements of the national legislation to which the undersigned is subject, transposing Annex IV section 4 of the Directive 98/79/EC on In Vitro Diagnostic Medical Devices. We certify that the design of the device(s) listed conforms with the relevant provisions of Annex IV section 4 of the directive 98/79/EC on In Vitro Diagnostic Medical Devices as transposed into national legislation.

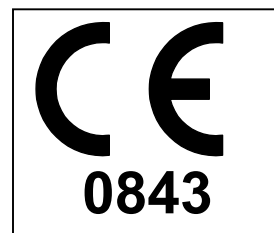
Certificate issued by:



Certification Manager
For UL International (UK) Ltd

UL International (UK) Ltd
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Certificate no: 601
Original certificate: 10 June 2009
Current certificate: 10 June 2009
Certificate expiry: 02 March 2013



PACKAGE INSERT

bioelisa HTLV-I+II 5.0

READ HIGHLIGHTED CHANGES

3000-1165

192 tests

ELISA test for the detection of antibodies to HTLV-I and HTLV-II in human serum or plasma. It is intended as a screening test, requiring repeat testing of initially reactive samples.

Summary

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 **bioelisa HTLV-I+II 5.0** is a direct sandwich immunoassay that utilises 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 had shown **bioelisa HTLV-I+II 5.0** is capable of detecting antibodies to HTLV-3/STLV-3 (see specific Performance Chapter).

The **bioelisa HTLV-I+II 5.0** is intended as a semi-quantitative enzyme-linked immunosorbent assay for the detection of antibodies to both HTLV-I and HTLV-II found in human serum or plasma. It is intended as a first line screen, requiring repeat testing of initially reactive specimens and confirmation of repeat reactive specimens by supplemental assays.

Principle

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 a coated well. 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 sample.

Components

- MCPL** MICROPLATE:
2 Plates of twelve 8-well strips. Each well contains adsorbed HTLV-I and-HTLV-II recombinant proteins. Store at 2-8°C.
- CONTROL-** NEGATIVE CONTROL:
1 x 1.8 ml of 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. Store at 2-8°C.
- CONTROL+** POSITIVE CONTROL:
1 x 1.8 ml of inactivated human serum containing a high titre of IgG antibodies specific for HTLV-I/II and non reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains thimerosal and sodium azide as preservative. Store at 2-8°C.

4. **DIL** DILUENT:
1 x 50 ml of phosphate buffered saline with casein and detergent. Contains Bronidox™ as preservative. Store at 2-8°C.
5. **WASH SOLN 20x** CONCENTRATE WASHING SOLUTION:
1 x 120 ml of phosphate buffered saline with Tween-20. Contains chloracetamide as preservative. Store at 2-8°C.
6. **CONJ** CONJUGATE:
1 x 160 µl of HTLV trifuision antigen labeled with horseradish peroxidase. Contains 0.02% thimerosal as preservative. Store at 2-8°C.
7. **SUBST TMB** SUBSTRATE-TMB:
1 x 25 ml of colourless buffer containing 3,3' 3,5' - tetramethylbenzidine (TMB). Store in the dark at 2-8°C.
8. **H₂SO₄ 2M** STOPPING SOLUTION:
1 x 30 ml of 2M sulphuric acid solution. Store at 2-8°C.
9. **SEALS** ADHESIVE SEALS:
To cover the microplate during incubations.
10. **BAG** RESEALABLE BAG:
For storage of unused strips.

* Bronidox is a Trade Mark of Henkel Chemical Co.

Precautions

bioelisa HTLV-I+II 5.0 is intended for IN VITRO diagnostic use.

For professional use only.

Please refer to the product labelling for information on potentially hazardous components.

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, POSITIVE AND NEGATIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS.** It is recommended that the components and test specimens be handled using good laboratory working practices. They should be disposed of in accordance with established safety procedures.

The **Positive Control** and **Negative Control** contain 0.005% thimerosal and 0.1% 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. The following are the appropriate risk (R) and safety (S) phrases.

Thimerosal:

R26/27/28 Very toxic by inhalation, in contact with skin and if swallowed.

S28-36-45 After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Sodium Azide:

R28-32 Very toxic if swallowed. Contact with acids liberates very toxic gas.

S28-45 After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

The **Diluent** contains 0.5% Bronidox™, which is classified pursuant to applicable European Economic Community (EEC) Directives as irritant (Xi). The following are the appropriate risk (R) and safety (S) phrases.

R22	Harmful if swallowed.
R38	Irritating to skin.
S36	Wear suitable protective clothing.
S46	If swallowed, seek medical advice immediately and show this container or label.

The **Concentrate Washing Solution (20x)** contains 2% chloroacetamide which is classified pursuant to applicable European Economic Community (EEC) Directives as Irritant (Xi). The following are the appropriate risk (R) and safety (S) phrases.

R25	Toxic if swallowed.
R43	May cause sensitization by skin contact.
R62	Possible risk of impaired fertility.
S22	Do not breathe dust.
S36/37	Wear suitable protective clothing and gloves.
S45	In case of accident or if you feel unwell, seek medical advice Immediately (show the label when possible).

The **Stopping Solution** is 2M Sulphuric acid which is classified pursuant to applicable European Economic Community (EEC) Directives as corrosive (C). The following are the appropriate risk (R) and safety (S) phrases.

R35	Causes severe burns.
S26-30-45	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Never add water to this product. In case of accident or if you feel unwell, seek medical advice immediately (Show label where possible).

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, Positive and Negative Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard 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-TMB 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. Serum or plasma samples collected in EDTA, Heparin, Sodium Citrate, K-Oxalate or Acid Citrate Dextrose (ACD) may be used. 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 Instruction Manual. 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 labels.
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 aliquot, 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 ($25^{\circ}\text{C} \pm 3^{\circ}\text{C}$) before use. Immediately after use return at 2-8°C 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 2-8°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.
17. Use of highly haemolysed 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 plates.
19. During 37°C incubation evaporation must be prevented. Cover plates with adhesive seals 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 **bioelisa HTLV-I+II 5.0** kit and its components at 2-8°C when not in use.
2. All test reagents and strips when stored at 2-8°C, are stable until the expiry date given on the kit. Do not freeze reagents.

- Crystals may form when Concentrate Washing solution (20x) is stored at 2-8°C. These must be dissolved by warming at 37°C prior to use.
- 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.
- Opened, unused microplate strips must be stored with the desiccant provided at 2-8°C in a closed pouch.

Collection of samples

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

Fresh samples are preferred, specimens that undergo freezethaw cycles repeatedly are not recommended. Samples should be stored 2-8°C if the test is to be run within 7 days of collection or frozen at -20°C 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 samples stored at 2-8°C.

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

- Loosen caps of serum containers.
- Heat specimen at 56°C for 30 minutes in a water bath.
- Allow serum to cool down before re-tightening caps.
- Serum can be stored frozen until analysis.

Repeated freeze-thawing of the sample is not recommended.

Material required not provided

- Disposable absorbent bench top paper and paper towels.
- Polypropylene tubes or containers.
- Graduated pipettes: 5 ml, 10 ml.
- Multichannel pipette capable of delivering 50 µl, 100 µl and 200 µl.
- Pipettes capable of delivering 1 - 1000 µl.
- Disposable pipette tips.
- Reagent reservoirs (troughs) with a capacity of 25 ml.
- Deionised or distilled water, reagent grade quality.
- Flasks: 500 ml, 1 litre.
- ELISA microplate washer. Alternatively, washing can be performed manually by using a multichannel pipette delivering 0.3 ml volumes and an aspirator device.
- A 37 ± 1°C incubator.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 630 nm is advisable.
- Effective disinfectant.

Automatic processing

Automated or semi-automated assay may be used with different instruments. It is very important to validate any automated system to demonstrate that results obtained for samples are equivalent to the ones obtained using manual assay. It is recommended that the user validate periodically the instrument. If there is any difficulty in the setting of Biokit automatic processors, please contact your distributor.

Preparation of reagents

1. WORKING CONJUGATE

- WORKING CONJUGATE should be **prepared fresh prior to use**.
- Mix conjugate and diluent thoroughly before use. **DO NOT SPIN** the conjugate vial.
- Dilute conjugate at 1:200 dilution factor with diluent. For example, add 10 µl conjugate into 2.0 ml diluent.
- Use only polypropylene containers or tubes.

CONJUGATE PREPARATION CHART (1:200 dilution factor)		
Number of tests	Vol. of conjugate (µl)	Vol. of diluent (ml)
24	15.0	3.0
48	25.0	5.0
72	30.0	6.0
96	40.0	8.0

2. DILUTED WASHING SOLUTION

- a. DILUTED WASHING SOLUTION is stable for 2 weeks at room temperature.
- b. Dilute 1 volume of CONCENTRATE WASHING SOLUTION with 19 volumes of distilled or deionised water (reagent grade quality). Mix well. Approximately 200 ml of washing solution is required to wash 1 plate.

PROCEDURE (See procedural flow chart)

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. Well A1 is 'BLANK'. DO NOT ADD SPECIMEN TO THIS WELL. Add 50 µl of diluent to this well. 50 µl
7. Add 50 µl of test specimen to the assigned well, starting at well H1. 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 are added. 50 µl
8. Add 50 µl of NEGATIVE CONTROL per well to wells B1, C1 and D1. Mix by pipetting up and down at least once. 50 µl
9. Add 50 µl of POSITIVE CONTROL per well to wells E1, F1 and G1. 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 an adhesive seal to prevent evaporation during incubation. -
11. Incubate for 60 ± 2 minutes at 37 ± 1°C. (Do not use a waterbath for incubation). 60 min
12. Remove and discard the adhesive seal 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. 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
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 another adhesive seal. | 100 µl |
| 15. Incubate for 30 ± 2 minutes in the dark at 37 ± 1°C. | 30 min |
| 16. Remove and discard the adhesive seal. | - |
| 17. Using a multichannel pipettor, add 50 µl of STOPPING SOLUTION 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 - 630 nm. | |

NOTE: Absorbance should be read within 10 minutes upon addition of the STOPPING SOLUTION.

Quality control

- Please ensure that each test specimen and control is properly mixed with WORKING CONJUGATE by pipetting up and down at least once after addition.
- Change in colour of WORKING CONJUGATE indicates that serum or plasma has been added.
- The BLANK should be assayed in one well, whereas NON-REACTIVE CONTROL and REACTIVE CONTROL in triplicates on each plate with each run of specimens.
- Blank values must have an absorbance of ≤ 0.100 .
- Negative Control values must have an absorbance of ≤ 0.100 after subtracting the blank value.
- At least 2 of the 3 Positive Control values must have absorbance ≥ 0.600 subtracting the blank value. Any values outside of this range should not be used for calculation of the Positive Control mean (PCx).
- For invalid assays, refer to TROUBLESHOOTING GUIDE.

Results

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

THE MEAN BLANK VALUE SHOULD BE SUBTRACTED FROM ALL ABSORBANCE VALUES ON THE PLATE BEFORE INTERPRETATION OF RESULTS.

The presence or absence of antibodies specific for HTLV-I/II is determined by relating the absorbance of the samples to the CUT-OFF VALUE (COV) of the plate.

The CUT-OFF VALUE is calculated by adding 0.250 to the mean absorbance of Negative Control:

$$\text{CUT-OFF VALUE} = 0.250 + \text{NCx}$$

Calculation of results

- Calculation of Negative Control mean absorbance (NCx).

Example:	Well No.	Absorbance
	B1	0.020
	C1	0.021
	D1	<u>0.022</u>
	Total	0.063

$$\text{Mean } 0.063 / 3 = 0.021 \text{ (NCx)}$$

Individual Negative Control values should be ≤ 0.100 .

If one Negative Control value does not meet the above criteria, it must be excluded as aberrant.

The Negative Control mean (NCx) should then be recalculated using the remaining individual Negative Control values. All remaining individual Negative Control values must meet the above criteria or the assay is invalid and must be repeated.

2. Calculation of Positive Control mean absorbance (PCx).

Example:	Well No.	Absorbance
	E1	1.221
	F1	1.144
	G1	<u>1.298</u>
	Total	3.663

Mean $3.663 / 3 = 1.221$ (PCx)

Individual Positive Control values must be ≥ 0.600 .

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

3. Calculation of CUT-OFF value.

CUT-OFF = $0.250 + NCx$

Example: $NCx = 0.021$ CUT-OFF = $0.250 + 0.021 = 0.271$

Interpretation of the results

1. Samples with absorbance values less than the CUT-OFF value are considered non-reactive.
2. Samples with absorbance values greater than or equal to the CUT-OFF value are considered initially reactive by the criteria of the **bioelisa HTLV-I+II 5.0** and should be retested in duplicate before interpretation.
3. Samples found reactive on retesting may be interpreted to be repeatedly reactive for antibodies to HTLV-I/II.
4. Initially reactive samples which are non-reactive on retesting are considered negative.
5. Samples which are repeatedly reactive in the **bioelisa HTLV-I+II 5.0** should be further tested by additional, more specific tests.

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	bioelisa HTLV-I+II 5.0			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% (4990/4999) for the random blood donor, and 100% for clinical samples and potentially interfering samples.

Table 2: Diagnostic Specificity of **bioelisa HTLV-I+II 5.0** in Various Groups of Samples.

Sample Group	bioelisa HTLV-I+II 5.0			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 **bioelisa HTLV-I+II 5.0** was evaluated in-house using three serum calibrators including a Positive Control (PC), a HTLV-I positive sample and a HTLV-II positive sample.

Inter-lot: 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).

Intra-lot: 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 **bioelisa HTLV-I+II 5.0**.

Samples	Assay Components	No. of Replicates	Mean OD/COV	Inter-lot Precision (CV %)	Intra-lot Precision (CV %)
Positive 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
	#2	30	8.073	13.6	
	#3	30	8.688	10.1	

Limitations of the procedure

A repeatedly reactive result with the **bioelisa HTLV-I+II 5.0** is presumptive evidence of antibodies to HTLV-I/II in the sample. A NON-REACTIVE result indicates the likely absence of detectable antibodies to HTLV-I/II in the sample. However, there is insufficient data to exclude the transmission of HTLV-I/II in blood samples determined to be non-reactive with the **bioelisa HTLV-I+II 5.0**. A NEGATIVE result therefore does not exclude the possibility of exposure to or infection with HTLV-I/II.

Falsely reactive results can be suspected with a test kit of this nature. The proportions of falsely reactive will depend on the sensitivity and the specificity of the test kit. For most screening assays, the higher the prevalence of HTLV-I/II antibody in a population, the lower the proportion of falsely reactive samples.

Limited expressed warranty disclaimer

The manufacturer makes no expressed warranty other than that the test kit will function as an IN VITRO diagnostic 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 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.

Technical problems

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 your local distributor.

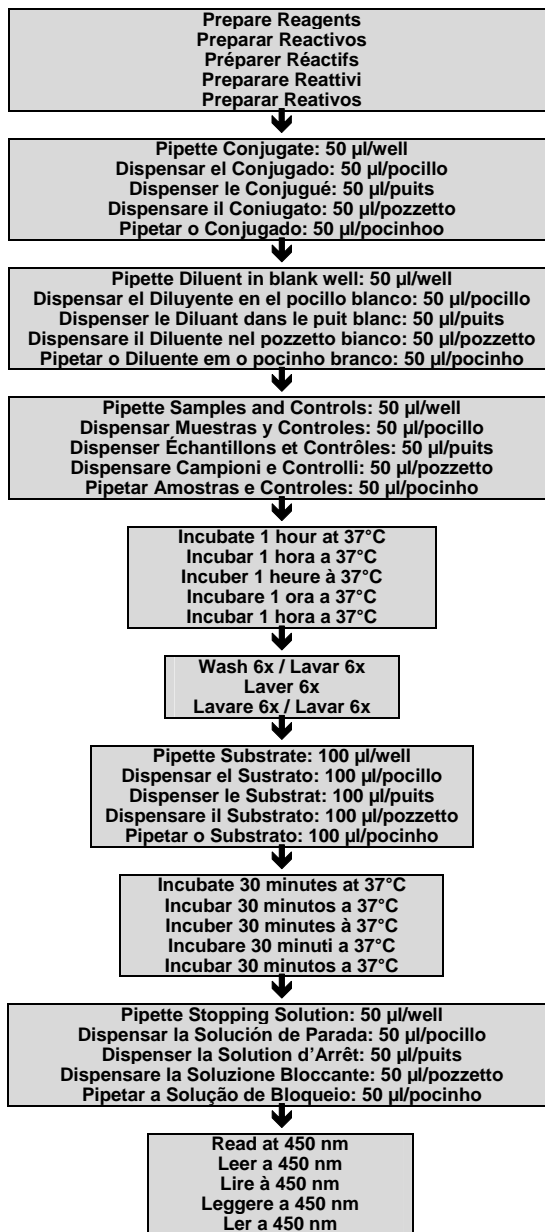
bioelisa: Troubleshooting guide

Problem	Possible causes	Solution
1. Controls out of validation.	1a. Incorrect temperature, incubation or pipetting.	<i>Check procedure. Repeat assay.</i>
	1b. Improper preparation of reagents, error of dilution, reagents not well mixed.	<i>Check procedure. Repeat assay.</i>
	1c. Cross-contamination of controls.	<i>Pipette carefully. Do not interchange caps. Repeat assay.</i>
	1d. Incorrect reading filter.	<i>Check that the wavelength of the filter used is 450 nm. If no reference filter of 620 - 630 nm is used, absorbance increases approximately 0.050.</i>
	1e. Interference in the optical pathway.	<i>Check the reader. Clean or dry the bottom of wells. Check for air bubbles. Repeat reading.</i>
	1f. Used components from different lots.	<i>Do not use components from different lots as they are adjusted for each batch released.</i>
	1g. Expired reagents.	<i>Check the kit expire date. Use a non- expired kit and reagents.</i>
2. No colour or only a light colour developed at the end of the assay.	2a. One or more reagents not added or added in wrong sequence.	<i>Check procedure. Repeat assay.</i>
	2b. Inactive conjugate: improper conservation.	<i>Check for contamination. Repeat assay.</i>
	2c. Inactive microplate: improper conservation.	<i>Always keep unused strips in the bag very well closed, with the desiccant inside. Repeat assay.</i>
	2d. Inactive substrate: improper conservation, the container used affects substrate stability, cross-contamination with the stopping solution.	<i>Use disposable containers or wash with acid or ethanol and rinse with deionised water before re-use. Check procedure. Repeat assay.</i>

bioelisa: Troubleshooting guide

Problem	Possible causes	Solution
3. Too much colour in all microplate wells.	3a. Contaminated or oxidised substrate.	<i>Use disposable containers or washed with acid or ethanol. Repeat assay.</i>
	3b. Contaminated washing solution (1x).	<i>Check the quality of distilled or deionised water used for dilution. Repeat assay.</i>
	3c. Insufficient washing or washing not consistent: filling volume and/or aspiration insufficient or not uniform. Insufficient number of washing cycles, contaminated device.	<i>Check the washer. Fill wells with washing solution close to the top, aspirate completely. Increase the number of wash cycles and soak time. After washing, blot the inverted microplate on tissue paper.</i>
	3d. Using of a washing solution from other manufacturer.	<i>Use only biokit washing solution.</i>
4. Poor reproducibility or high number of non-repeatable reactive samples.	4a. Washing problems.	<i>See 3c, 3d, 3e.</i>
	4b. Uncalibrated pipettes or tips not well fitted. Improper pipetting.	<i>Use only calibrated pipettes, with well-fitted tips and pipette carefully, without bubbles and splashing. Repeat assay.</i>
	4c. Reagents and sera not at room temperature or not well mixed before using.	<i>Equilibrate reagents and sera to room temperature and mix thoroughly before using.</i>
	4d. Air currents over the microplate during incubations.	<i>Keep the microplate protected from air currents.</i>
	4e. Too long time for addition of samples and/or reagents. Inconsistency in time intervals. Air bubbles.	<i>Develop consistent and uniform technique.</i>
	4f. Interference in the optical pathway.	<i>See 1e.</i>

Procedural flow chart / Esquema del procedimiento / Schéma de la procédure /
Schema del procedimento / Esquema do procedimento



Cut-off: NCx + 0,250
 Valor umbral: CNx + 0,250
 Valeur seuil: CNx + 0,250
 Valore di soglia: CNx + 0,250
 Cut-off: CNx + 0,250

SAFETY DATA SHEET

BIOKIT S.A. 08186 Lliçà d'Amunt (Barcelona) Spain Phone: (34) 93 860 9000 Fax: (34) 93 860 9029 e-mail: biokit@biokit.com	SAFETY DATA SHEET	Date: 18-09-09 Rev.: 00 Pag.: 1 of 5
	Code: 3000-1165	

1. Identification of preparation
Product name: bioelisa HTLV-I+II 5.0

2. Composition/information on ingredients
Kit components: Microplate: Each well contains adsorbed HTLV-I and HTLV-II recombinant proteins. Conjugate: HTLV trifusion antigen labeled with horseradish peroxidase. Contains 0,02% thimerosal as preservative. Diluent: Phosphate buffered saline with casein and detergent. Contains Bronidox™ as preservative. Positive control: Inactivated human serum containing a high titre of IgG antibodies specific for HTLV-I/II and non reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains thimerosal and sodium azide as preservative. Negative 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. Concentrate washing solution: Phosphate buffered saline with Tween-20. Contains chloracetamide as preservative. Substrate-TMB: Colourless buffer containing 3,3',5,5'-Tetramethylbenzidine (TMB). Stopping solution: 2 M sulphuric acid solution.

3. Hazards identification						
Chemical characterisation						
Component	Dangerous components/ingredient according to EC-criteria					
	Ingredient name	CAS No.	EINECS No.	Hazard Classification	Risk Code	Concentration (% by weight)
Microplate	Not applicable	n.a.	n.a.	n.a.	n.a.	n.a.
Positive and negative controls	Sodium azide	26628-22-8	247-852-1	T+; Very Toxic N; Dangerous for the Environment	R 28-32-50/53	≤ 0.1 %
	Thimerosal	54-64-8	200-210-4	T+; Very Toxic N; Dangerous for the Environment	R 26/27/28-33-50/53	≤ 0.1 %
Diluent	Bronidox-L	30007-47-7	250-001-7	Xi; Irritant	R36/38	≤ 0.5 %
Concentrate washing solution	Chloroacetamide	79-07-2	201-174-2	T; Toxic	R25-43-62	≤ 2 %
Conjugate	Not applicable	n.a.	n.a.	n.a.	n.a.	n.a.
Substrate (TMB)	3,3',5,5'-Tetramethylbenzidine	54827-17-7	259-364-6	n.a.	n.a.	≤ 0.05 %
Stopping solution	2M Sulphuric acid	7664-93-9	231-639-5	C; Corrosive	R35	10%

4. First aid measures
Inhalation: Remove from exposure. If breathing is difficult, obtain medical attention if necessary. Eye contact: Rinse immediately with water for at least 15 minutes separating eyelid. Skin contact: Wash off thoroughly with plenty of water. Remove and wash contaminated clothing. Ingestion: Obtain medical attention

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5. Fire-fighting measures

Extinguishing media: Use media in adaptation to materials stored in the immediate neighbourhood.
Special firefighting procedures: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

6. Accidental release measures

After spillage: Wipe up spills with inert absorbant tissues and place in a suitable container. Use protective gloves.

7. Handling and storage

Handling: The normal precautions for handling chemicals must be observed. Wash hands after handling.
Storage: Between +2°C to 8°C.

8. Exposure controls/personal protection

Respiratory protection: Good local ventilation.
Eye protection: Protective glasses.
Hand protection: One-way gloves (latex or plastic).
 Use protective lab coat. Is recommended the use of safety pipette device.

9. Physical and chemical properties

Appearance:

Microplate: 2 Plates of twelve 8-well strips.
 Conjugate: colourless liquid.
 Diluent: green liquid.
 Positive control: red liquid.
 Negative control: yellowish liquid.
 Concentrate washing solution: colourless liquid.
 Substrate-TMB: colourless liquid.
 Stopping solution: colourless liquid.

Odour: Odourless

Boiling point: N/A

Melting point: N/A

Flash point: N/A

Ignition temperature: N/A

Explosion limits: N/A

Vapour pressure: N/A

Density: N/A

Viscosity: N/A

Solubility in water: soluble

10. Stability and reactivity

Conditions and materials to avoid: N/A
Hazardous reactions: N/A
Hazardous decomposition products: N/A

11. Toxicological information

Data not available.

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12. Ecological information

Negative and positive control/Conjugate-Readily biodegradable.

Substrate (TMB)/Diluent/Concentrate washing- Data not available.

Stop solution- Do not allow undiluted product or large quantities of it to reach ground water, water bodies or sewage system.

13. Disposal considerations

Remains of samples, reagents and controls should be collected in a recipient for this purpose and autoclaved 1 hour at 121°C.

Observe all Federal, State and local laws.


14. Transport information

Specials requirements: None


15. Regulatory information

According to 1999/45/EC Directive and 91/155/EEC Directive and following modifications.


Positive and negative controls:

Hazard symbol	Xn	Harmful	
R-phrases R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.		
S-phrases S13 S28 S36 S45 S60 S61	Keep away from food, drink and animal feeding stuffs. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing and gloves. In case of accident or if you fell unwell, seek medical advice immediately. This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/Safety data Sheet.		


Diluent:

Hazard symbol	Xi	Irritant	
R-phrases R22/38	Harmful if swallowed and irritating to skin.		
S-phrases S36/46	Wear suitable protective clothing. If swallowed, seek medical advice immediately and show this container or label		

Concentrate washing solution:

Hazard symbol	Xi	Irritant	
R-phrases R25-43-62	Harmful if swallowed. May cause sensitization by skin contact. Possible risk of impaired fertility		
S-phrases S22-36/37-45	Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show label when possible).		

Stopping Solution:

Hazard symbol	C	Corrosive	
R-phrases R35	Causes severe burns.		
S-phrases S26-30-45	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Never add water to this product. In case of accident or if you feel unwell, seek medical advice immediately (Show label where possible).		

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16. Other information

Intended use: ELISA test for the detection of antibodies to HTLV-I and HTLV-II in human serum or plasma. It is intended as a screening test, requiring repeat testing of initially reactive samples.

For IN VITRO diagnostic use.

Some reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Use proper disposal procedures.

Each donor unit used in the preparation of the controls of this kit was tested by an approved method for the presence of the antibodies to HIV and HCV as well as for HBsAg and found to be negative. **WARNING:** Because no test method can offer complete assurance that HIV, HCV, HBsAg or other infectious agents are absent, the controls of this kit should be handled carefully.

The above information is believed to be correct, but does not purpose to be all inclusive and should be used only as a guide. BIOKIT S.A. shall not be held for any damage resulting from handling or use of the product.