MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line HTLV-1 & HTLV-2 IgG is a qualitative test for the detection of IgG antibodies against human T-cell lymphotropic virus (HTLV) in human serum or plasma. The test enables differentiation between virus types 1 and 2.

2 Field of application

The *recom*Line HTLV-1 & HTLV-2 IgG is a line immunoassay. The separate line-up of individual antigens means that, unlike ELISA, the test allows identification of specific antibodies against the individual antigens of HTLV. The following recombinant antigens are used in the test: to differentiate between the two HTLV types, the antigens GAG-p19, GAG-p24 and ENV-gp46 are used. ENV-gp21 is used as a type-independent marker.
The *recom*Line HTLV-1 & HTLV-2 IgG is a confirmatory test and is used to verify the results of HTLV screening tests.

3 Test principle

Highly purified recombinant antigens are fixed to nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample with specific antibodies binding to the pathogen antigens on the test strips.
2. Unbound antibodies are then washed away.
3. The strips are incubated in a second step with anti-human immunoglobulin antibodies (IgG) that are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then washed away.
5. Specific bound antibody is detected in a colour reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band appears on the strip at the corresponding location.

Control bands are located at the top end of the test strip:

a) The reaction control below the strip number for which every serum/plasma sample must show a reaction.
b) The conjugate control (IgG) is used to check the antibody class detected. If the test strips are used to identify IgG antibodies, the IgG conjugate control band shows a clear band.
c) 'Cut-off control': The intensity of this band enables an evaluation of the reactivity of the individual antigen bands (see 9.2 Analysis).

4 Reagents

4.1 Package contents

The reagents in one pack are sufficient for 20 assays.
Each set of reagents contains:

DILUBUF	**100 ml** dilution buffer (**ready to use**) Contains Tris buffer, NaCl, detergent, preservative MIT (0.01%) and Oxypyrion (0.1%) and protein
WASHBUF A 10 X	**100 ml** wash buffer A (**10× concentrate**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml** chromogenic substrate tetramethylbenzidine (**TMB, ready-to-use**)
MILKPOW	**5 g** low-fat milk powder
INSTRU	**1** instructions for use
EVALFORM	**1** evaluation sheet
TESTSTR	**2** tubes each with 10 consecutively numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate (**100× concentrate, green cap**) From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)
CONTROL + IgG	**140 µl** positive serum control IgG (**red cap**) Human origin, anti-HCV, anti-HIV-1/2 and HBs-Ag negative, contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgG	**140 µl** negative serum control IgG (**blue cap**) Human origin, anti-HCV, anti-HIV-1/2 and HBs-Ag negative, contains MIT (0.1%) and Oxypyrion (0.1%)

4.2 Additional reagents required – equipment required

- Incubation trays (can be purchased from MIKROGEN as required)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotatory device
- Vacuum pump or similar device
- Measuring cylinders, 50 ml and 1000 ml
- Micropipettes with single-use tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Single-use protective gloves
- Waste container for biohazardous substances

5 Shelf life and handling

- Store reagents between +2°C and +8°C before and after use; **do not freeze**.
- Before starting the test, allow all reagents to sit at room temperature (+18°C to +25°C) for at least 30 minutes. The test is carried out at room temperature and all reagents used must be at room temperature.
- Wash buffer A, milk powder, dilution buffer, conjugates and TMB can be exchanged between the different *recom*Line and/or *recom*-Blot test systems if these components carry the same symbol. In doing so, pay attention to the shelf life of these components.
- Before use, mix the concentrated reagents and patient sera thoroughly. Avoid foam formation.
- Only open the tubes with the test strips just before use to prevent water condensation. Strips that are not needed must be left in the tube and are continued to be stored at +2°C to +8°C (reseal tube firmly, test strips must not be moist before the start of the test!). **Test strips can only be used for a maximum of 9 months after opening the test kit!**
- The strips are identified with a consecutive number and a test abbreviation.
- The packages have an expiry date, after which no further guarantee of quality can be given.
- Keep the kit components away from direct sunlight throughout the test procedure. The substrate solution (TMB) is light sensitive.
- The test may only be performed by trained, authorised and qualified personnel.
- Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by MIKROGEN.
- Cross-contamination of the patient samples or conjugates in the kit can lead to false test results. Add patient samples, test strips and conjugate solution carefully. Make sure that any incubated liquids are not carried over to other wells. Carefully remove liquids.
- The strips must be completely wetted and submersed throughout the entire procedure.
- Automation is possible. Further details are available from MIKROGEN.

6 Warnings and safety precautions

- Only use for *in-vitro* diagnostics.
- All blood products must be treated as potentially infectious.
- The test strips were manufactured with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasitic antigens.
- After adding patient or control material, the strips must be considered to be potentially infectious and handled appropriately as such.
- Suitable single-use gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide (NaN_3), MIT (methylisothiazolinone), Oxypyrion and chloroacetamide. Avoid contact with the skin or mucous membranes. Sodium azide (NaN_3) can form explosive azides if it comes into contact with heavy metals such as copper and lead.
- All aspirated liquids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens or be autoclaved. All reagents and materials that come into

contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentrations and incubation times specified by the manufacturer must be followed.
- Only use incubation trays once.
- Handle strips carefully with a pair of plastic forceps.
- Do not replace or mix the reagents with reagents of other manufacturers.
- Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and reagent preparation

7.1 Sample material

The sample material can be either serum or plasma (EDTA, citrate, heparin, CPD) and must be separated as soon as possible after collection to avoid haemolysis. Microbial contamination of the sample must absolutely be avoided. Non-soluble substances must be removed from the sample prior to incubation. Use of icteric, haemolytic, lipaemic or cloudy samples is not recommended.

Caution!

If the tests are not carried out immediately, the sample material can be stored for up to 2 weeks between +2°C and +8°C. It is possible to store the samples for longer periods at −20°C or lower. Repeated freezing and thawing of the samples is not recommended due to the risk of false results. More than 3 freezing and thawing cycles should be avoided.

7.2 Preparation of solutions

7.2.1 *Preparation of the ready-to-use wash buffer A*

This buffer is required for the serum and conjugate dilution and the wash steps.

Before the dilution, the volume of wash buffer A must be determined for the corresponding number of tests to be carried out.

The low-fat milk powder is first pre-dissolved in wash buffer A concentrate and then this mixture is made up to the final volume with deionised water (dilution 1 + 9). The required quantity for a defined number of test strips is calculated using the following formulae (device-specific dead volumes are not taken into account):

Reagent	Formula	Example: 5 strips
Low-fat milk powder [g]	= strip number × 0.1	0.5 g
Wash buffer A concentrate [ml]	= strip number × 2	10 ml
Deionised water [ml]	= strip number × 18	90 ml
Ready-to-use wash buffer A [ml]	= strip number × 20	100 ml

Ready-to-use wash buffer A can be stored at +2°C to +8°C for four weeks. The ready-to-use wash buffer A has no odour and is slightly turbid.

7.2.2 *Preparation of conjugate solutions*

The conjugate solution must be prepared shortly before use; the ready-to-use conjugate solution must not be stored.

One part of the conjugate concentrate is diluted with 100 parts of the ready-to-use wash buffer A (1 + 100).

The required quantity for a defined number of test strips is calculated using the following formulae:

Reagent	Formula	Example: 5 strips
Conjugate concentration [µl]	= strip number × 20	100 µl
Ready-to-use wash buffer A [ml]	= strip number × 2	10 ml

The conjugate quantities are calculated without dead volumes. Depending on the processing (manually or with a device), please prepare additional conjugate solution for 1 to 3 strips.

8 Test procedure

No.	Implementation	Note
1	Before starting the test, allow all reagents to sit at +18°C to +25°C (room temperature) for at least 30 minutes.	The test is carried out at room temperature and all reagents used must be at room temperature.
2	Prepare the test strips Place strips in **2 ml** of ready-to-use **dilution buffer**.	Do not handle the strips with bare hands – use forceps. The strip number faces upwards. For each strip one well in an incubation tray (see 4.2) is required. The strips must be completed submersed.
3	Sample incubation	
a)	**20 µl** of an undiluted sample (human serum or plasma) or control are added by pipette to the test strip for each incubation mixture (dilution 1 + 100).	Pipette the sample/control onto one end of the submersed strip in dilution buffer and mix as quickly as possible by gently shaking the incubation bath.
b)	Incubate for **3 hours** with gentle shaking.	Cover the incubation tray with the plastic lid and place on the shaker.
4	Wash	Carry out wash steps 8.4a to 8.4c a total of three times.
a)	Carefully remove the plastic lid from the incubation tray.	Avoid cross-contamination.
b)	Carefully aspirate the serum dilution from the individual wells.	With automated processing, follow the directions of the device manufacturer for this step.
c)	Pipette **2 ml** ready-to-use **wash buffer A** (see 7.2.1) into each well, wash for 5 minutes with gentle shaking and then aspirate off the wash buffer A.	
5	Incubation with conjugate Add **2 ml** ready-to-use **conjugate solution** (see 7.2.2) and incubate for **45 minutes** with gentle shaking.	Cover the incubation tray with the plastic lid and place on the shaker.
6	Wash (see point 8.4)	Carry out wash steps a total of three times (see 8.4a to 8.4c).
7	Substrate reaction Add **1.5 ml substrate solution** and incubate for **8 minutes** with gentle shaking.	
8	Stopping the reaction Remove the substrate solution. Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips Dry the strips before the analysis for **2 hours** between 2 layers of absorbent paper.	Carefully remove the strips from the water with a pair of plastic forceps. Store the strips protected from light.
Caution! **Incubation solutions must not be carried over to other wells. Avoid splashing, particularly when opening and closing the cover.**		

9 Results

Caution:

Do not use the automated interpretation without noting the information described below about the interpretation.

9.1 Validation – Quality control

The test can only be analysed when the following criteria are satisfied:

1. Reaction control band (upper line): clearly stained, dark band.
2. Antibody class (second band): the IgG conjugate control band must be clearly stained.
3. Cut-off control (third band): weak but visible staining.

Negative and positive controls are not necessary for analysing the test. They can be included if needed for internal quality control.

The controls must have the following reactive antigen bands:

- Positive control: The two ENV antigens gp21 and gp46-1 must be reactive. All other antigens may react but do not have to.
- Negative control: None

9.2 Analysis

The test strips can be analysed visually or with a computer using the *recom*Scan test strip analysis software. The *recom*Scan software is intended to help with test strip interpretation. Additional information and corresponding instructions for computer-aided analysis are available from MIKROGEN upon request. The following instruction refers to the visual analysis.

9.2.1 *Evaluation of the band intensity*

1. Note the date and batch number along with the antibody class that was detected in the attached evaluation sheet.
2. Enter the sample identification numbers in the evaluation sheet.
3. Now adhere the associated test strip with a glue stick into the corresponding field in the evaluation sheet. Align the test strip with the reaction control band at the indicated marker line. Then adhere the test strip to the left of the marking line using transparent adhesive tape (do not stick over the reaction control band!). Adhering the entire test strip with glue stick or adhesive tape can lead to changes in the staining.
4. Now identify the bands for the developed test strips using the printed control strip from the evaluation sheet and enter these into the evaluation sheet. Carry out the evaluation of the intensity of the emergent bands separately for the relevant immunoglobulin classes using Table 1.

Table 1: Evaluation of the band intensity relative to the cut-off band

Colour intensity of the bands	Evaluation
No reaction	-
Very weak intensity (less than the cut-off band)	+/-
Weak intensity (corresponds to the cut-off band)	+
High intensity (greater than the cut-off band)	++
Very high intensity	+++

9.3 Interpretation of the test results

The test result is determined by the addition of the point values as described in Table 2 for the individual reactive bands that are ≥ cut-off (that is, evaluated as at least +). The resultant sum is entered in the column with the sum symbol.
The positive, borderline or negative evaluation of the sample can then be determined directly with Table 3 and entered in the evaluation sheet in the column Evaluation.
It must be noted that for the reaction of the p19, p24 and gp46 bands, the point value is only calculated once, regardless of whether only one type or both types react.

Table 2: Points evaluation of the antigens

Antigen	Points IgG
p19	1
p24	1
gp46	2
gp21	2

Table 3: Test interpretation

Sum of points	Evaluation of IgG
≤ 1	Negative
2	Borderline
≥ 3	Positive

The differentiation is made using the antibody reactivity to the structural proteins (GAG) p19 and p24 as well as to the envelope protein (ENV) gp46. **Differentiation is only possible if the test result is positive (that is, the sum is at least 3 points)** (see Tables 2 and 3).

Table 4: Differentiation

Differentiation	Criteria*
HTLV-1	• gp46-1 reacts ≥ cut-off and gp46-1 has a significantly higher intensity than gp46-2 or • both gp46 react with the same intensity ≥ cut-off or both gp46 react < cut-off and p19-1 reacts ≥ cut-off
HTLV-2	• gp46-2 reacts ≥ cut-off and gp46-2 has a significantly higher intensity than gp46-1 or • both gp46 react with the same intensity ≥ cut-off or both gp46 react < cut-off and p19-1 reacts < cut-off and p24-2 reacts ≥ cut-off
Cannot be typed	• Test result is positive and • neither the criteria for HTLV-1 nor HTLV-2 apply

*The reactivity of the antigens p19-2 and p24-1 are not included in the differentiation.

10 Limits of the method, restrictions

- Serological test results must always be viewed within the context of other medical assessments of the patient. The therapeutic consequences of the serological finding must be related to the clinical data.
- A negative test result cannot rule out infection with the human T-cell lymphotropic virus. In the early stage of the infection, antibodies may not be present or may be present in undetectable amounts. If an infection with HTLV is suspected, another sample should be collected and tested after three to eight weeks.
- Patients with borderline results should be tested again in two to six weeks in all cases. For additional verification, it is recommended to perform an RT-PCR test to detect the HTLV genome.
- The correlation between positive antibody detection and infectiousness is not possible.
- Dark test strips: Some patient samples can produce a dark generalised or patterned staining over the entire nitrocellulose strip (e.g. with sera from patients with milk protein allergies). There are a number of different factors associated with the patient serum that are responsible for this. Evaluating these strips is generally only possible with limitations. Such ‘inverse’ bands (white bands on a dark background) must be rated negatively. The corresponding serum should be checked using another serological method.
- The presence of weak bands that have an intensity that is clearly below that of the cut-off is possible in rare cases but does not influence the performance of the test.

11 Performance characteristics

11.1 Diagnostic sensitivity

*recom*Line HTLV-1 & HTLV-2 IgG	HTLV-1 (n = 206)	HTLV-2 (n = 110)
Negative	0	0
Borderline	2	7
Positive	204	103
Sensitivity	(2+204)/206 = 100%*	(7+103)/110 = 100%**

*Including two borderline results.
**Including seven borderline results.

11.2 Differentiation between HTLV-1 and HTLV-2

*recom*Line HTLV-1 & HTLV-2 IgG	HTLV-1 (n = 204) only the positives from the 206 samples	HTLV-2 (n = 103) only the positives from the 110 samples
Positive to HTLV-1	204	0
Positive to HTLV-2	0	101
Differentiation not possible	0	2
Correct differentiation	204/204 = 100%*	101/103 = 98.1%**

*181 classifications using the gp46-1/gp46-2 ratios; additional 23 using p19-1 ≥ 1 COI.
*99 classifications using the gp46-2/gp46-1 ratios; additional 2 using p24-2 ≥ 1 COI.

11.3 Diagnostic specificity

Blood donors, clinical and interfering samples:

*recom*Line HTLV-1 & HTLV-2 IgG	Blood donors (n = 200)	Clinical samples* (n = 239)	Potentially interfering samples** (n = 80)
Negative	193	235	79
Borderline	7	4	1
Positive	0	0	0
Specificity	193/200 = 96.5%	235/239 = 98.3%	79/80 = 98.8%

*Samples from patients with acute hepatitis, early EBV infection, ANA-positive autoimmune diseases, pregnant women and samples from bacterial laboratory routine.
**Lipaemic, haemolytic and icteric samples, RF-positive samples.

11.4 HIV infections and HIV/HTLV dual infections

a) HIV-1: A total of 19 HIV-1 positive / HTLV-negative samples from Central Europe were tested, each of which showed an HTLV-negative result. A further 5 samples with confirmed HIV-1/HTLV dual infection from French Guiana, Guadeloupe or Martinique were tested, each of which showed an HTLV-positive result.

b) HIV-2: A total of 21 HIV-2-positive samples from the Ivory Coast were tested. Of these, 17 confirmed HIV-2 positive / HTLV-negative samples showed an HTLV-negative result. Furthermore, 2 samples with confirmed HIV-2/HTLV dual infection showed a positive and a borderline HTLV result. In addition, 2 samples with suspected HIV-2/ HTLV dual infection each showed a borderline HTLV result.

11.5 Analytical specificity

The analytical specificity is defined as the ability of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) Interference: Control studies on potentially interfering factors have shown that the performance of the test is not influenced by anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia, bilirubinaemia or three freeze/thaw cycles of the sample.

b) Cross-reactions: Potential interference of antibodies against other organisms were investigated in control studies with similar clinical symptoms as seen with infection with HTLV (e.g. EBV and viral hepatitis) as well as an infection with related pathogens (HIV, HCV). In addition, conditions were tested that can be attributed to atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor). No cross-reactivity was detected (see 11.3).

12 Literature

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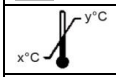
We will be pleased to send you additional literature on HTLV diagnosis upon request.

13 Explanation of symbols

Σ	Content is sufficient for <n> formulations Number of formulations
DILUBUF	Dilution buffer
WASHBUF A 10 X	Wash buffer A (10× concentrate)
SUBS TMB	Chromogenic substrate tetramethylbenzidine
MILKPOW	Low-fat milk powder
INSTRU	Instructions for use
EVALFORM	Evaluation sheet
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
CONTROL + IgG	Positive serum control IgG
CONTROL - IgG	Negative serum control IgG
	Follow the instructions for use
CONT	Contents, contains
IVD	*In-vitro* diagnostic agent
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store between x°C and y°C
	Manufacturer

14 Manufacturer and version data

***recom*Line HTLV-1 & HTLV-2 IgG**	Article no. **5272**
Instructions for use Valid from	GARLHT004EN 2023-02

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