



Instructions for use

1 Purpose

RESEARCH USE ONLY!

2 Field of application

The *recomLine* 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.

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 color 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) are 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	100ml dilution buffer (ready to use) Contains Tris buffer, NaCl, detergent, preservative: MIT (0.01%) and Oxypyrion (0.1%) and protein.
WASHBUF A (10 X)	100ml wash buffer A (10× concentrate) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	40ml chromogenic substrate tetramethylbenzidine (TMB, ready-to-use)
MILKPOW	5g 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	500µl anti-human IgG conjugate (100× concentrate, green cap) From rabbit, contains NaN ₃ (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)
CONTROL + IgG	140ul 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	140ul 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 Additionally 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, 50ml and 1000ml
- Micropipettes with single-use tips, 20µl and 1000µl
- 10ml 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 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 *recomLine* and/or *recomBlot* 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 samples 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!).
- ☞ 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 must 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 samples or conjugates in the kit can lead to false test results. Add 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 submerged throughout the entire procedure.
- ☞ Automation is possible. Further details are available from MIKROGEN.

6 Warnings and safety precautions

- ☞ Only for *in vitro* research purposes.
- ☞ 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 samples 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 (NaN₃), MIT (methylisothiazolinone), Oxypyrion and chloroacetamide. Avoid contact with the skin or mucous

- membranes. Sodium azide (NaN₃) 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 deionized 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	0.5g
Wash buffer A concentrate [ml]	= strip number × 2	10ml
Deionised water [ml]	= strip number × 18	90ml
Ready-to-use wash buffer A [ml]	= strip number × 20	100ml

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	100µl
Ready-to-use wash buffer A [ml]	= strip number × 2	10ml

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	<u>Prepare the test strips</u> Place strips in 2ml 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 completely submersed.
3	<u>Sample incubation</u> a) 20µl 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). b) Incubate for 3 hours with gentle shaking.	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. Cover the incubation tray with the plastic lid and place on the shaker.
4	<u>Wash</u> a) Carefully remove the plastic lid from the incubation tray. b) Carefully aspirate the serum dilution from the individual wells. c) Pipette 2ml 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.	Carry out wash steps 8.4a–8.4c a total of <u>three times</u> . Avoid cross-contamination. With automated processing, follow the directions of the device manufacturer for this step.
5	<u>Incubation with conjugate</u> Add 2ml 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	<u>Wash</u> (see point 8.4)	Carry out wash steps a total of <u>three times</u> (see 8.4a–8.4c).
7	<u>Substrate reaction</u> Add 1.5ml substrate solution and incubate for 8 minutes with gentle shaking.	
8	<u>Stopping the reaction</u> Remove the substrate solution. Wash at least three times briefly with deionized water .	
9	<u>Drying the strips</u> 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) is 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 analyzing 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 as well as none or 1 or 2 of the GAG antigens p19-1 and p24-1.
- Negative control: None

9.2 Analysis

The test strips can be analysed visually or with a computer using the *recomScan* test strip analysis software. The *recomScan* 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

- Note the date and batch number along with the antibody class that was detected in the attached evaluation sheet.
- Enter the sample identification numbers in the evaluation sheet.
- 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.
- 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

Color 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 $\geq$ 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
gp-46	2
gp-21	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 $\geq$ cut-off <u>and</u> gp46-1 has a significantly higher intensity than gp46-2 <u>or</u> both gp46 react with the same intensity $\geq$ cut-off or both gp46 react $<$ cut-off <u>and</u> p19-1 reacts $\geq$ cut-off
HTLV-2	- gp46-2 reacts $\geq$ cut-off <u>and</u> gp46-2 has a significantly higher intensity than gp46-1 <u>or</u> both gp46 react with the same intensity $\geq$ cut-off or both gp46 react $<$ cut-off <u>and</u> p19-1 reacts $<$ cut-off <u>and</u> p24-2 reacts $\geq$ cut-off
Cannot be typed	Test result is positive <u>and</u> neither the criteria for HTLV-1 nor HTLV-2 apply

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

11 Explanation of symbols

	Content is sufficient for <n> formulations Number of formulations
DILUBUF	Dilution buffer
WASHEUF A 10 X	Wash buffer A (10x 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
RUO	Research Use Only
LOT	Batch number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store between x °C and y °C
	Manufacturer

12 Manufacturer and version data

recomLine HTLV-1 & HTLV-2 IgG	Article no. 5272
Instructions for use Valid from	GARLHT002EN 2021-02
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