MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line HIV 1 & HIV 2 IgG is a qualitative test for the detection of IgG antibodies against the human immunodeficiency virus 1 (HIV 1) as well as HIV 2 in human serum or plasma.

2 Intended use

The *recom*Line HIV 1 & HIV 2 IgG is a line immunoassay. By separately lining up the individual antigens, unlike ELISAs, the test principle allows the identification of specific antibodies against the individual antigens of HIV-1 and HIV-2 (ENV proteins HIV-1: gp120, gp41; ENV proteins HIV-2: gp105, gp36; GAG proteins: p24, p17; POL proteins: p51, p31).
By using the type-specific antigens gp41 (HIV 1) and gp36 (HIV 2) it is also possible to differentiate between an infection with HIV 1 and HIV 2.
The *recom*line HIV-1 & HIV-2 IgG is a confirmation test and can be used to clarify unclear screening results.

3 Test principle

Highly purified recombinant HIV antigens are fixed on nitrocellulose membrane strips.

1. The test strips are incubated with the diluted serum or plasma sample, with specific antibodies attached to the pathogen antigens on the test strip.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG), which are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:

a) The reaction control band under the strip number, which must show a reaction for every serum/plasma sample.
b) The conjugate control band (IgG) is used to check the detected antibody class. If, for example, the test strip is used for the detection of IgG antibodies, the IgG conjugate control will show this clearly on the band.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 20 tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.1%)
SUBS TMB	**40 ml** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form
TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate (**hundred times concentrated, green screw cap**) From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)
CONTROL + IgG	**140 µl** positive serum control IgG (**red screw cap**) Human origin, anti-HCV and HBs Ag negative, contains MIT (0.1%) and oxypyrion (0.1%)
CONTROL - IgG	**140 µl** negative serum control IgG (**blue screw cap**) Human origin, anti-HCV, anti-HIV 1/2 and HBs Ag negative, contains MIT (0.1%) and Oxypyrion (0.1%)

4.2 Materials required but not supplied

- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling

- Store reagents at +2 °C to 8 °C before and after use, **do not freeze**.
- Subject all ingredients for at least 30 minutes to room temperature (+18 °C to 25 °C) before beginning the test. The test procedure is carried out at room temperature.
- Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different *recom*Line and *recom*Blot test systems, if these components carry the same symbols. Consider the shelf life of these components.
- Mix the concentrated reagents and samples thoroughly before use. Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2 °C to +8 °C (reseal tube tightly, test strips must not become moist before the test!).
- The strips are marked with the serial number, as well as the test code.
- The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In case of significant changes by the user to the product and/or the instructions for use, application may be beyond the purpose specified by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells. Carefully drain liquids.
- The strips must be completely wetted and immersed throughout the entire procedure.
- Automation is possible; you will receive further information from MIKROGEN.

6 Warnings and precautions

- For *In vitro* diagnostic use only.
- All blood products must be treated as potentially infectious.
- The test strips were manufactured with inactivated whole cell lysates and / or recombinant produced bacterial, viral or parasitic antigens.
- After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion and chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
- All siphoned liquids must be collected. All containers must include appropriate disinfectants for the inactivation of pathogenic human viruses and other pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.

- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and carefully follow the directions. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling, so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation.
The use of heat-inactivated, icteric, haemolysed, lipemic or turbid samples is not recommended.
Caution!
If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at 2 °C to 8 °C. Prolonged storage of the samples is possible at -20 °C or below. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results. Avoid more than 3 cycles of freezing and thawing.

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*
This buffer is required for serum and conjugate dilution as well as washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First, the skimmed milk powder is dissolved in wash buffer A concentrate, and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

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

Ready to use wash buffer A can be stored at 2 °C – 8 °C for up to four weeks. The ready to use wash buffer A is odourless and easily marred.

7.2.2 *Preparation of conjugate solutions*
The conjugate solution must be prepared immediately before use. It is not possible to store the ready for use conjugate solution.
One part of the conjugate concentrate is diluted with 100 parts of the ready to use wash buffer A (1 + 100).
The quantities required for a defined number of test strips are to be calculated according to the following formula:

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

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

No.	Execution	Note
1	Temper all reagents for at least 30 minutes at 18°C - 25° (room temperature) before beginning the test.	The test procedure is carried out at room temperature.
2	Prepare test strips Place the strips in **2 ml** of ready-to-use **wash buffer A**.	Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) or control is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **3 hours** with gentle shaking	 Pipette the sample/control at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray. Cover the incubation tray with plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. Pipette **2 ml** of ready to use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	Carry out washing stages 8.4a-8.4c three times in total. Avoid cross-contamination The manufacturer's instructions must be followed during automatic processing.
5	Incubation with conjugate Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** with gentle shaking.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see under 8.4	Carry out washing stages three times in total (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** of ready-to-use **substrate solution** and incubate for **8 minutes** while shaking gently.	
8	Stopping the reaction Wash at least three times **briefly** with **deionisied water**.	
9	Drying the strips Dry the strips between 2 layers of absorbent paper for **2 hours** prior to analysis.	Carefully remove strips from water using plastic forceps. Store strip away from light.
Caution! **Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.**		

9 Results

Caution:
Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation – Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:
1. Reaction control band (top line) with clearly visible stain, dark band
2. Antibody class (second band): the IgG conjugate control band must show a clear staining.
3. Cut-off control (third band): weak, but visible staining

Negative and positive controls are not required for evaluating the test. They may be carried out for internal quality control purposes where necessary.

The controls must have the following reactive antigen bands:
Positive control: gp120, gp41, p51, p31, p24, p17;
gp105 and gp36 may react, but need not react.
Negative control: no

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software *recom*Scan. The *recom*Scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*
1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.
2. Enter the sample identification numbers to the evaluation sheet.
3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Now identify the bands of the developed test strips using the printed control strip on the evaluation sheet and enter it into the log sheet. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	Assessment
No reaction	-
Very low intensity (lower than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
High intensity (higher than the cut-off band)	++
Very strong intensity	+++

9.3 Interpretation of test results

The criteria for interpreting the test are to be taken from Table 2.

Table 2: Test Interpretation

Test result	Criteria
Negative	No bands ≥ cut-off or gp120 and/or gp105 ≥ cut-off
Borderline	Any band constellation that does not fulfil the criteria for negative or positive
Positive	Two ENV bands of the same HIV type (gp120 + gp41 or gp105 + gp36) ≥ cut-off or one ENV band (only gp41 or gp36) and at least one GAG band (p17, p24) or POL band (p31, p51) ≥ cut-off

Differentiation is by means of the transmembranal glycoproteins gp41 (HIV-1) and gp36 (HIV-2) and is only possible in the case of positive test results (see tables 2 and 3),

Table 3: Differentiation

Differentiation	Criteria
HIV 1	• The test result is positive and • gp41 reacts ≥ cut-off and • gp41 reacts significantly stronger than gp36
HIV 2	• The test result is positive and • gp36 reacts ≥ cut-off and • gp36 reacts significantly stronger than gp41
non-typeable	• The test result is positive and • neither the criteria for HIV-1 nor for HIV-2 apply.

10 Limitations of the method - restrictions

- When the positive findings are based entirely on an evaluation of two glycoprotein bands of the same HIV type (gp120+gp41 and/or gp105+gp36), a further sample should be tested two to four weeks later. As an additional precaution, an (RT) PCR test is recommended for the HIV genome.
- Patients with questionable results should be tested again after two to four weeks under all circumstances. As an additional precaution, an (RT) PCR test is recommended for the HIV genome. According to the literature, test results are often unclear in pregnant women.
- A negative test result cannot exclude an infection with the human immunodeficiency viruses. In the early phase of infection antibodies may not yet be present or not present in a detectable quantity. If infection with HIV is suspected a further sampling and testing should be carried out after two weeks.
- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- No correlation between positive antibody detection and infectiousness is possible.
- Dark test strips: Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip (e.g. on serums from patients with milk protein allergies). Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Diagnostic sensitivity

*recom*line HIV-1 & HIV-2 IgG	HIV 1* (n=238)	HIV 2* (n=104)
Negative	0	0
Borderline	0	1
Positive	238	103
sensitivity	238/238=100%	1+103/104=100%**

* including samples of subtypes A, B, C, D, F, G, CRF01, CRF02 from group M and group O.
** including one borderline result.

11.2 Differentiation between HIV 1 and HIV 2

*recom*line HIV-1 & HIV-2 IgG	HIV 1 (n=238)	HIV 2 (n=103)
Positive to HIV-1	233	0
Positive to HIV-2	0	101
Differentiation not possible	5	2
Correct differentiation	233/238=98%	101/103=98%

11.3 Seroconversions

Fifteen seroconversions panels with a total of 147 inspections were performed with the *recom*Line HIV 1 and HIV 2 in direct comparison with another commercially available confirmatory test. In two panels the *recom*Line HIV 1 & HIV 2 antibodies detected antibodies against HIV earlier than the comparative test. In one other panel the *recom*-Line HIV 1 & HIV 2 were reactive one inspection later. In the remaining twelve seroconversion panels both confirmation tests detected the HIV antibodies at the same time.

11.4 Diagnostic specificity

*recom*line HIV 1 & HIV 2 IgG	Blood donors (n=300)	Clinical samples* (n=340)	Potentially interfering samples** (n=56)
Negative	298	336	54
Borderline	2	4	2
Positive	0	0	0
Specificity	298/300=99,3%	336/340=98,8%	54/56=96,4%

* Samples from patients with acute hepatitis, recent EBV infection and various autoimmune diseases, pregnant women and samples from the laboratory routine.
** Lipemic, haemolytic and icteric samples, RF-positive samples, patients with hypergammaglobulinemia.

11.5 Analytical specificity

The analytical specificity is defined as the capacity 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) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (citrate, EDTA, heparin, CPD), haemolysis (up to 1000 mg/dl haemoglobin), lipaemia, bilirubinaemia (up to 20 mg/dl bilirubin) or three cycles of freezing and thawing do not affect the performance of the test.

b) Cross-reactions: Potential interferences of antibodies against other organisms, as described in the literature regarding cross-reactivity in HIV confirmation tests (e.g. EBV, acute viral hepatitis) were examined in control studies. Additionally, conditions that are attributed to atypical activity of the immune system (antinuclear autoantibodies, rheumatoid factor) were tested. No cross-reactivities were detected (see 11.4).

12 Literature

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7. Soutschek E, Höflacher B, Motz M. 1990. Purification of a recombinantly produced transmembrane protein (gp41) of HIV I. J Chromatogr. Nov 23;521(2):267-77.
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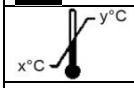
We will gladly send you further literature on the diagnosis of HIV upon request.

13 Explanation of symbols

	Content is sufficient for <n> applications Number of applications
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*line HIV-1 & HIV-2 IgG**		Item no. **6672**
Instructions for use valid from		GARLHI007EN 2023-02
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