*recom*Line Treponema IgG
*recom*Line Treponema IgM

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

*recom*Line Treponema IgG, IgM is an immunoassay for the qualitative determination of IgG and IgM antibodies to Treponema pallidum in human serum or plasma.

2 Intended use

The *recom*Line Treponema IgG, IgM can be used as a confirmatory test in the screening of reactive samples.
The *recom*Line Treponema IgG, IgM is a line immunoassay. In contrast to ELISAs, this testing principle allows the separate lining up of individual antigens and thus the determination of specific antibodies to individual Treponema pallidum antigens. The test uses recombinantly-produced antigens: Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17 and Tp15.

3 Test principle

Highly-purified, recombinant Treponema antigens are fixed on nitrocellulose membrane strips.

1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG and /or IgM), 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 is located under the strip number, and must demonstrate a reaction for each serum/plasma sample.
b) The conjugate controls (IgG, IgM) are used for the inspection of conjugate and strip types (Ig-class specific). If the IgG-specific test strip is used for the detection of IgG antibodies, the IgG conjugate control band will show a strong reaction; while the IgM-specific test must show positive reactivity in the IgM control 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 (100) tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml (5x100 ml)** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml (5x40 ml)** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g (5x5 g)** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1 (5)** Evaluation form

4.1.1 *recomLine Treponema IgG*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (10)** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl (5x500 µl)** anti-human IgG conjugate **(100-fold concentration, green cap)** From rabbit, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.2 *recomLine Treponema IgM*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (10)** tubes, each with 10 numbered test strips
CONJ IgM	**500 µl (5x500 µl)** anti-human IgM conjugate **(hundred times concentrated, purple screw cap**) From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.2 Additional reagents, materials and devices required

- 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.
- Where different *recom*Line and *recom*Blot tests are used, the same reagents (see printed symbol) can be used across the whole range of parameters and batches. The shelf life of these components should be noted.
- 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 may 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 substantial changes to the product or the regulations for use by the user, the application may lie outside the purpose given 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 remove liquids.
- The strips must be completely wetted and submerged throughout the entire procedure.
- Automation is possible; further information can be obtained 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 prepared with inactivated bacterial or viral 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 collecting containers must contain suitable disinfectants for inactivation of human 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 them. 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 before incubation.
The use of heat-inactivated, icteric, haemolytic, lipemic or turbid samples is not recommended.
Caution!
If tests are not carried out immediately, samples can be stored for up to 2 weeks at +2 to +8 °C. More extended storage of the samples is possible at -20 °C or lower. Repeated freezing and thawing of samples is not recommended due to the risk of inaccurate results.

7.2 Preparation of solutions

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

This buffer is required for sample and conjugate dilutions, as well as for the washing stages.
The volume of wash buffer A for the corresponding number of tests must be determined before dilution.
First of all, 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 for up to 4 weeks at +2 C to +8°C. The ready-to-use wash buffer A is odourless and slightly turbid.

7.2.2 *Preparation of conjugate solutions*

The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.
One part of the conjugate concentrate is diluted with 100 parts of 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

8.1 One hour of serum incubation

No.	**Execution**	**Note**
1	Subject all reagents for at least 30 minutes to 18°C - 25°C (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**. **Important:** IgG and IgM strips are not interchangeable!	Do not touch the strips with bare hands - use the forceps. The strip number points upward. Place each strip in a separate well in the incubation tray (see 4.2).The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 Pipette the sample 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 a 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** ready for use **wash buffer A** in every 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 all. Avoid cross-contamination. The manufacturer's instructions must be observed during automatic processing.
5	Incubation with conjugate Add **2 ml** 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 8.4	Carry out the washing stages three times in all (see 8.4a-8.4c).
7	Substrate reaction Add **1.5 ml** ready-to-use **substrate solution** und incubate for **8 minutes** with gentle shaking.	
8	Stopping the reaction Remove substrate solution Wash at least three times **briefly** with **deionisied water**.	
9	Drying the strips Dry strip between 2 layers of absorbant paper for **2 hours** before 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.**		

8.2 Three hours of serum incubation

Alternatively, the test can be performed with three hours of serum incubation, with the only difference to the procedure described under 8.1. being points 3a) and 3b).

3 a) b)	Incubation of samples **10 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 200) Incubate for **3 hours** with gentle shaking.	 Pipette the sample 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 a plastic cover and place in the shaker.

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 (uppermost line) is clearly stained, dark band
2. Antibody class (second band): the IgG and/or IgM conjugate control band must show clear staining.
3. Cut-off control (third band): weak, but visible staining

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 in 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 strip on the basis of the printed control strip of the evaluation sheet and enter this in the evaluation sheet. For this purpose, carry out the assessment of the intensity of the occurring bands on the basis of Table 1 separately for the corresponding immunoglobulin classes.

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 weakly cutoff band)	+/-
Low intensity (equivalent to cutoff band)	+
Strong intensity (stronger than cutoff band)	++
Very strong intensity	+++

9.3 Interpretation of test results

Please see Table 2 for the test interpretation criteria.

Table 2: Test interpretation

Test result	Criteria
Negative	no antigen ≥ cut-off
Borderline	only one random antigen ≥ cut-off
Positive	at least two random antigens ≥ cut-off

10 Limitations of the method - restrictions

- 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.
- Discussing possible cross-reactions is important for the interpretation of the test results. Like the genus Borrelia, the genus Treponema is part of the Spirochaetaceae family. The literature describes cross-acting antibodies to partial antigens that are common to the Spirochaetaceae family (4).
  Cross-reacting antibodies to antigens TP47, TmpA, Tp257 (Gpd), Tp453, TP17 and TP15 as used in *recom*Line Treponema have not been described. They are characteristic Treponema pallidum antigens that show no reactivity to Borrelia-positive sera.
- A negative test result for *recom*line Treponema cannot exclude an infection with Treponema pallidum. Further sampling and testing should be performed after four weeks with existing, clinical suspicion of infection with Treponema pallidum and negative, serological results.
- Positive IgG and/or IgM results are not always an indication for an active disease process.
- Dark test strips:Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip. 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 Treponema**	**Earlier positive findings in two reference tests**			
	1 hour processing		**3 hours processing**	
	IgG (n=280)	**IgM (n=90)**	**IgG (n=39)**	**IgM (n=38)**
Negative	0	0	0	0
Borderline	2	7	1	0
Positive	278	83	38	38
Sensitivity	**100%***	**100%***	**100%***	**100%**

* including inconclusive results.

11.2 Diagnostic specificity

***recom*Line Treponema**	**Blood donor**			
	1 hour processing		**3 hours processing**	
	IgG (n=200)	**IgM (n=199)**	**IgG (n=40)**	**IgM (n=40)**
Negative	199	196	40	38
Borderline	1	3	0	2
Positive	0	0	0	0
Specificity	**99,5%**	**98,5%**	**100%**	**95%**

11.3 Analytical specificity

The analytical specificity is defined as the capacity of the test to determine the analytes exactly 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 (CPD, sodium citrate, EDTA, heparin), haemolysis (up to 1,000 mg/dl haemoglobin), lipaemia, bilirubinaemia (up to 20 mg/dl bilirubin) or cycles of freezing and thawing do not affect the performance of the test.
b) Cross-reactions: The potential interferences of antibodies with other organisms (Borrelia burgdorferi, HCV, HIV) have been investigated in control studies. Also tested were conditions caused by atypical activitiy of the immune system (antinuclear autoimmune antibodies, rheumatoid factor, pregnancy, fresh herpes simplex infection e.g. EBV*) are examined. No cross reactions were shown.
* Acute EBV infections may cause a nonspecific IgM reactivity in the *recom*Line Treponema IgM (e.g. polyclonal stimulation).

12 Literature

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Further Information on Treponema diagnostics is available on request.

13 Explanation of symbols

Σ	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
y°C x°C	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line Treponema IgG** ***recom*Line Treponema IgM**	Item. No. **5172 (5170)** Item. No. **5173 (5179)**
Instructions for use valid from	GARLTP004EN 2023-03

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