*recom*Bead Treponema IgG 2.0
*recom*Bead Treponema IgM 2.0

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Bead Treponema IgG 2.0, IgM 2.0 is a qualitative in vitro test for the detection of IgG and IgM antibodies against Treponema pallidum in human serum and plasma.

2 Field of application

*recom*Bead Treponema 2.0 can be used as a confirmatory test for positive or unclear samples from screening.
Based on individual antigens immobilised on specifiable microparticles, *recom*Bead Treponema 2.0 enables reliable identification of specific antibodies against selected Treponema antigens.

2.1 Licence information

With the acquisition of the IVD (in vitro diagnostic device) *recom*Bead Treponema 2.0, based on MagPlex® microparticles from Luminex®, the client agrees to the licence condition stipulated by Luminex® that this test or its components are used exclusively in association with the Luminex100, Luminex200 or MAGPIX® analysis systems.

3 Test principle

Ultrapure recombinant Treponema antigens (Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17 and Tp15) are separately coupled to various magnetic microparticles (beads) with different fluorescence coding. Antibodies against individual Treponema antigens are detected in parallel in a single solution. Detection of the antibodies corresponds to the Immunoblot test principle.

1. The antigen particles are incubated with the diluted serum or plasma sample with specific antibodies binding to the pathogen antigens on the microparticles.
2. Unbound antibodies are then washed away.
3. In a second step, the microparticles are incubated with anti-human immunoglobulin antibodies (IgG or IgM), which are coupled to R-Phycoerythrin (PE).
4. Unbound conjugate antibodies are then washed away.
5. Specifically bound antibodies are detected by the fluorescence of R-Phycoerythrin when excited by light. If an antigen-antibody reaction has occurred, the typical fluorescence appears on the surface of the microparticles and can be measured with the Luminex analysis system.

In addition to the antigen-coated microspheres, four other bead populations that act as controls are included in the mixture:

a) An incubation control, which must display a reaction in every solution upon addition of serum or plasma.
b) A conjugate control, which must display a reaction in each individual sample upon addition of conjugate.
c) A conjugate control, which distinguishes IgG or IgM conjugate.
d) A negative control, which must not exceed a defined threshold when the test is performed.

4 Reagents

4.1 Package contents

The reagents of one pack are sufficient for 96 tests.
Each test kit contains:

DILUBUF 6X	**100 ml** wash and dilution buffer (**six-fold concentration**) Contains Tris buffer, NaCl, detergent, preservative MIT (0.06%) and Oxypyrion (0.6%) and protein
MTP	**2** 96-well microtitre plates
TAPE	**3** sealing films
INSTRU	**1** instructions for use
LOTCERT	**1** batch certificate with cut-off values

4.1.1 *recomBead Treponema IgG 2.0*

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

BEADMIX	**5.5 ml** microparticle suspension, coated with recombinant **Treponema** antigens (**ready-to-use**, **black** cap), contains preservatives methylisothiazolone (MIT, 0.01%) and Oxypyrion (0.1%)
CONJ IgG	**5.5 ml** anti-human IgG-conjugate (**ready-to-use**, **green** cap) From goat, contains Proclin (0.1%)

4.1.2 *recomBead Treponema IgM 2.0*

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

BEADMIX	**5.5 ml** microparticle suspension, coated with recombinant **Treponema** antigens (**ready-to-use**, **black** cap), contains preservatives methylisothiazolone (MIT, 0.01%) and Oxypyrion (0.1%)
CONJ IgM	**5.5 ml** anti-human IgM-conjugate (**ready-to-use**, **red** cap) From goat, contains Proclin (0.1%)

4.2 Additionally required reagents, materials and equipment

- Deionised water (high quality)
- Measuring cylinder
- ELISA washer with magnetic base plate
- Equipment for setting up the serum dilutions (e.g. micro and multipipettes, 8-channel pipette 10 – 100 µl, masterblock or reaction containers, vortexer, plate shaker)
- Incubator (37°C)
- Luminex100, Luminex200 or MAGPIX® analysis system (incl. IS or xPONENT software, system accessories and system fluid)
- *recom*Quant-Software from Mikrogen incl. parameter-specific template for IS- or xPONENT software
- Timer
- Disposable protective gloves
- Waste receptacle for biohazardous substances
- If required, positive and negative controls are available from MIKROGEN. These controls are not necessary for implementing and evaluating the test.

5 Shelf life and handling

- Store reagents away from light at +2°C to +8°C before and after use, **do not freeze**.
- Before starting the test, let all reagents sit at room temperature (18–25°C) for at least 30 minutes.
- The same components (wash and dilution buffers, conjugate) from different *recom*Bead 2.0 tests can be used across parameters and batches. In doing so, attention must be paid to the shelf life of these components.
- Mix the patient samples thoroughly before use.
- The container with the particle mixture (Beadmix) must be vortexed thoroughly immediately prior to each use (30 – 60 sec, max. rpm), in order to attain an even suspension.
- Conjugates and particle mixtures that are not needed must be left in the tube and continue to be stored at +2°C to +8°C (reseal tube firmly).
- 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.
- Unused wells can be covered with sealing film.
- The test must only be performed by trained and authorised qualified personnel.
- If substantial changes are made by the user to the product or the directions for use, usage may be beyond the intended purpose specified by Mikrogen.

6 Warnings and safety precautions

- Only use for in vitro diagnostics.
- MIKROGEN has not validated these tests for screening of blood, blood components, cells, tissues, organs or any of their derivatives in order to assess the suitability for transfusion, transplantation or cell administration.
- All blood products must be treated as potentially infectious.
- Suitable disposable protective gloves must be worn during the entire test procedure.
- The conjugates contain Proclin (0.1%). The wash and dilution buffers and the particle mixtures contain MIT (methylisothiazolone) and CMIT (chloromethylisothiazolinone). Avoid contact with the skin or mucous membranes.
- The Luminex100, Luminex200 or MAGPIX® analysis system requires system fluid from a reservoir and discharges this as waste after the reading. Clear separation of supply and waste container must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly.
- All aspirated fluids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens. All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentration specifications and incubation times of the manufacturer must be taken into consideration.
- Individual wells of the microtitre plate must only be used once.
- Do not replace or mix the reagents with reagents of other manufacturers.
- Read through and carefully follow the entire instructions for use before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and preparation of reagents

7.1 Sample material

The sample material can be serum or plasma (CPD, EDTA, citrate, heparin). Serum and plasma should be separated from the blood clot as quickly as possible after removal, 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 heat-inactivated, 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 at +2°C to +8°C. Longer storage of the samples is possible 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 the ready-to-use wash and dilution buffer

This buffer is required for the washing steps and for the sample dilution.
The buffer concentrate is diluted **1 + 5** with deionised water.
24 ml of ready-to-use buffer (4 ml concentrate + 20 ml deionised H_2O) are prepared for one 8-well microtitre plate strip.
Ready-to-use wash and dilution buffers can be stored at +2°C to +8°C for four weeks.

8 Test procedure

No.	Implementation	Note
1	Before starting the test, let all reagents sit at room temperature (18–25°C) for at least 30 minutes.	
2	Preparation of the samples (and, where applicable, controls) Prepare **500 µl buffer**, add **10 µl non-diluted sample**.	Prior to use, the sample must be pre-diluted **1 + 50**. Controls can be ordered separately, they are not necessary for the test evaluation. Mix thoroughly!
3	Preparation of the particle mixture The particle mixture is mixed thoroughly (30 – 60 sec, max. rpm).	Vortex the particle mixture (Beadmix) before every use, to guarantee a homogeneous suspension.
4 a) b)	Preparation of the microtitre plate The required strips of the microtitre plate are equilibrated with **50 µl** buffer per cavity. Empty the wells by aspiration.	
5 a) b) c) d)	Sample incubation **50 µl** of the resuspended **particle mixture** are placed in the equilibrated wells. **50 µl** of the **diluted samples** are added per well. The samples and the particle mixture are mixed for 30 sec on a shaker at 900 rpm. Incubate for **20 minutes** at **37°C**.	At least one value is created for each control and patient sample.
6 a) b) c)	Wash (5x) Empty the wells by aspiration on a **magnetic** plate. Pipette **200 µl** buffer into each well and incubate for 30 – 60 sec. Empty the wells by aspiration on a **magnetic** plate.	**Caution, the microtitre place must not be tapped out!** The microtitre plate must lie correctly on the magnetic plate! Perform wash steps 8.6b – 8.6c a total of **five times**. The microtitre plate must lie correctly on the magnetic plate!
7	Incubation with conjugate Add **50 µl conjugate solution**, mix for 30 sec on the shaker at 900 rpm and incubate for **20 minutes** at **37°C**.	
8 a) b) c)	Wash (3x) Empty the wells by aspiration on a **magnetic** plate. Pipette **200 µl** buffer into each well and incubate for 30 – 60 sec. Empty the wells by aspiration on a **magnetic** plate.	**Caution, the microtitre place must not be tapped out!** The microtitre plate must lie correctly on the magnetic plate! Perform wash steps 8.8b – 8.8c a total of **three times**. The microtitre plate must lie correctly on the magnetic plate!
9 a) b) c)	Exposure to system fluid Empty the wells completely by aspiration on a **magnetic** plate. Fill up **each of** the wells **with 100 µl** ready-to-use **system fluid** of the analysis system. Mix the microtitre plate thoroughly on the shaker (30 sec, 900 rpm).	**Caution, the microtitre place must not be tapped out!** The microtitre plate must lie correctly on the magnetic plate! Homogeneous particle suspension important for reading on the Luminex analysis system.
10 a) b)	Measuring the fluorescence The fluorescence intensities on the individual particle surfaces are measured on the Luminex100, Luminex200 or MAGPIX® analysis system. Evaluation of the data by means of the evaluation program *recom*Quant.	Equipment and software operation according to the Luminex handbook. Software operation according to *recom*Quant operating instructions.

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 evaluated when the following criteria are fulfilled:

1. The incubation control must be positive. If the signal strength is insufficient, an error message is issued in the evaluation program *recom*Quant ("no serum").
2. The conjugate control must be positive. If the signal strength is insufficient, an error message is issued in the evaluation program *recom*Quant ("no conjugate").
3. The negative control must be negative. If a defined signal strength is exceeded, an error message is issued in the evaluation program *recom*Quant ("nc invalid").
4. A defined number of particles must have been evaluated from all used particle regions (corresponding to the attached antigens). If fewer particles were determined in the prescribed time interval, an error message occurs in the evaluation program *recom*Quant ("insufficient beads").

If one of these error messages occurs, testing of the sample is not valid and the sample is not evaluated.

9.2 Evaluation

The fluorescence intensities (MFI values) determined for the antigen reactivities of each sample are compared to a batch-specific reference value. The cut-off index (COI) is calculated from this ratio and a scaling factor. This calculation is carried out automatically in the *recom*Quant analysis program.

Evaluation of the individual antigen reactivities is summarised in the following table:

Table 1: Evaluation of fluorescence intensity (signal strength) in relation to the limit value

Signal strength	Evaluation
clearly below the limit value (COI < 0.67)	negative
below the limit value, but reaches at least 2/3 of the limit value (0.67 ≤ COI < 1.00)	borderline
equal to or above the limit value (COI ≥ 1.00)	positive
very clearly above the limit value (COI is no longer calculated)	highly positive

9.3 Interpretation of the test results

The test result is obtained by adding the corresponding point values in the case of positive and borderline results for individual antigens (Table 2).
The sum of the points resulting from the antigen reactivities is calculated automatically in the evaluation program. The positive, unclear or negative evaluation of the sample is directly displayed by *recom*Quant in accordance with Table 3.

Table 2: Points evaluation of the antigens

Antigen	Points IgG	Points IgM	Points borderline IgG/IgM
Tp47	2	2	1
TmpA	1	2	0
Tp257	1	1	0
Tp453	1	1	0
Tp17	2	2	1
Tp15	2	2	0

Table 3: Test interpretation

Sum of Points	Evaluation
< 2	negative
2	unclear
> 2	positive

10 Limits of the method, restrictions

- Serological test results must always be considered in context of the clinical picture. Therapeutic consequences of the serological finding must be connected with clinical data.
- When interpreting the test results, it is important to consider possible cross-reactions. The Treponema genus is, like the Borrelia genus, a member of the Spirochaetaceae family. The literature describes cross-reacting antibodies against partial antigens that are common to the Spirochaetaceae family (4).
  Cross-reacting antibodies against the Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17 and Tp15 antigens used in *recom*Bead Treponema 2.0 are not described. These are characteristic Treponema pallidum antigens with no reactivity to Borrelia-positive sera.
- A negative *recom*Bead Treponema 2.0 test result cannot rule out infection with Treponema pallidum. In case of existing clinically suspected infection with Treponema pallidum and negative serological results, further sampling and testing should be carried out again after four weeks.
- Positive IgG and/or IgM results are not always an indication for an active disease process.
- Polyclonal stimulation of B-lymphocytes can occur in the case of infectious mononucleosis (glandular fever, EBV infection). This can lead to non-specific reactions when detecting IgM class antibodies. It is recommended to rule out EBV infection by differential diagnosis in the case of unclear anamnesis and presence of a weak IgM response.

11 Performance characteristics

11.1 Diagnostic sensitivity

	Positive preliminary findings in two reference tests			
	IgG		IgM	
***recom*Bead Treponema 2.0**	**Preliminary finding**	***recom*Bead Tp**	**Preliminary finding**	***recom*Bead Tp**
Negative	0	0	0	0
Unclear	0	0	0	2
Positive	240	240	28	26
Sensitivity		**100%**		**100%***

*including the unclear results

11.2 Diagnostic specificity

	Blood donor**			
	IgG		IgM	
***recom*Bead Treponema 2.0**	**Preliminary finding**	***recom*Bead Tp**	**Preliminary finding**	***recom*Bead Tp**
Negative	294	294	296	288
Unclear	0	0	0	8
Positive	0	0	0	0
Specificity		**100%**		**97.3%**

**The analysed blood donor sera (blood donor service is the Bavarian Red Cross) are certified as non-reactive for Treponema.

11.3 Analytical specificity

The analytical specificity is defined as the suitability 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 the test performance is not influenced by anticoagulants (sodium citrate, EDTA, heparin, CPD) and haemolysis of the sample. False positive results occurred in the IgM detection for icteric and lipaemic sera and sera from patients with hyper-IgG syndrome. Repeated freezing and thawing of the samples can affect the test result.
b) Cross-reactions: Potential interference of antibodies against other organisms (Borrelia burgdorferi, CMV, HIV, HCV) was investigated in control studies. In addition, conditions were tested that can be attributed to atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, pregnancy, recent herpesvirus infection, e.g. EBV). In the IgM detection very few cross-reactivities with RF-positive sera were detected. With the exception of cross-reactivities of EBV-IgM-positive sera no other cross-reactivities were observed in the IgG detection.

12 Literature

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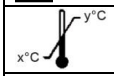
We will be pleased to send you additional literature on Treponema diagnosis upon request.

13 Explanation of symbols

	Contents are sufficient for <n> formulations Number of formulations
DILUBUF 6X	Wash and dilution buffer (six-fold concentration)
MTP	96-well microtitre plates
TAPE	Sealing film
INSTRU	Instructions for use
LOTCERT	Batch certificate
BEADMIX	Microparticle suspension
CONJ IgG	Anti-human IgG conjugate
CONJ IgM	Anti-human IgM conjugate
	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*Bead Treponema IgG 2.0** ***recom*Bead Treponema IgM 2.0**		Article no. **5154** Article no. **5155**
Instructions for use Valid from		GARXTP005EN 2023-05
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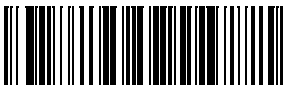
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