*recom*Well Parvovirus B19 IgG
*recom*Well Parvovirus B19 IgM

Instructions for use (English)

1 Purpose

The *recom*Well Parvovirus B19 IgG, IgM test is a qualitative and quantitative in-vitro test to detect and identify IgG or IgM antibodies against Parvovirus B19 in human serum or plasma. The *recom*Well Parvovirus B19 IgG, IgM is a screening test based on the principle of an indirect sandwich ELISA. RF absorption is recommended for handling the IgM samples.

2 Intended use

Parvovirus B19 causes Erythema infectiosum. A Parvovirus B19 infection during pregnancy may result in spontaneous abortion, still birth or Hydrops fetalis in sero-negative pregnant women. Parvovirus B19 IgG antibodies are retained for life after contact with the virus. Parvovirus B19 IgM-antibodies can be detected at the earliest approx. 10 days after contact with the virus. The *recom*Well Parvovirus B19 can be used as a screening test for determining the IgG- and IgM-antibody status. Positive and borderline results can subsequently be confirmed in a specific confirmation test (*recom*Line Parvovirus B19 IgG [avidity], IgM.

3 Test principle

Highly purified Parovirus B19 antigens (a specific part of the VP1 produced by recombination as well as recombinant, eukaryote VP2 particles) are fixed in the wells of the microtitre plate.

1. Diluted serum or plasma samples are incubated in the wells, with antibodies binding specifically to the pathogen antigens coating the surface of the wells.
2. Unbound antibodies are then flushed away.
3. In a second step, anti-human immunoglobulin antibodies (IgG and / or IgM), which are coupled to horseradish peroxidase, are incubated in the wells.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected by a peroxidase-catalysed colour reaction. If an antigen/antibody-reaction occurs, the chromogen substrate solution colours proportionally to the quantity of the bound anti-Parvovirus B19 IgG or IgM antibodies. The intensity of the staining can be measured with a photometer and then conclusions can be drawn concerning the concentration of the anti-Parovirus B19 antibodies in the sample.

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 96 tests.
Each test kit contains:

WASHBUF 10 X	**100 ml** Wash buffer (**ten times concentration**) Contains phosphate buffer, NaCl, detergent, preservative: MIT (0.01%) and Oxypyrion (0.1%)
DILUBUF	**125 ml** Dilution Buffer (**ready-to-use**) Contains protein, detergent and blue dye. Preservative: MIT (0.01%) and Oxypyrion (0.1%)
SUBS TMB	**12 ml** Chromogenic substrate tetramethylbenzidine (TMB, **ready-to-use**)
SOLN STOP	**12 ml** stop solution 24.9% phosphoric acid (H_3PO_4) (**ready for use**)
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form
TAPE	**2 pieces** of covering film

***recom*Well Parvovirus B19 IgG** also contains:

MTP	**12X8 wells** Microtitre plate (cap strip marked in **red**) coated with recombinant Parvovirus B19 antigen in a self-sealing vacuum bag.
CONTROL + IgG	**150 µl** positive control (**violet** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgG	**150 µl** cutoff control (**yellow** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgG	**150 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgG	**250 µl** anti-human IgG conjugate (**101-times concentrated**, **red** cap) contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

***recom*Well Parvovirus B19 IgM** also contains:

MTP	**12X8 wells** Microtitre plate (cap strip marked in **green**) coated with recombinant Parvovirus B19 antigen in a self-sealing vacuum bag.
CONTROL + IgM	**150 µl** positive control (**black** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgM	**150 µl** cutoff control (**colourless** cap) contains MIT (0.1%) and oxypyrion (0.1%)
CONTROL - IgM	**150 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgM	**250 µl** anti-human IgG conjugate (**101-times concentrated**, **green** cap) contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

4.2 Materials required but not supplied

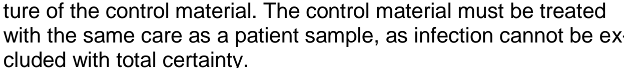
- Deionised water (high quality)
- Test tube
- Vortex mixer or other rotators
- 8-channel pipette or washer with pump
- Clean measuring cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 10 µl and 1000 µl
- 10 ml pipette or dispenser
- Incubation chamber 37 °C
- Microtitre plate photometer
- Timer
- Disposable protective gloves
- Waste container for bio-hazardous materials
- Mikrogen recommends processing the IgM samples by measuring their RF absorption with rheumatoid factor absorbent (Siemens Item No.: OUCG15). The "Recommendations for the performance of the RF absorption test" can be requested from MIKROGEN. See also 8.2b.

5 Shelf life and handling

- Store reagents at +2 °C to +8 °C before and after use, **do not freeze**.
- Subject all ingredients to room temperature (+18 to +25 °C) for at least 30 minutes before beginning the test.
- The components dilution buffer, wash buffer, substrate and stop solution for the *recom*Well test can be used across the whole range of parameters and batches. At the same time, the shelf life of these components is to be noted.
- The control serums and conjugates are batch-dependent and may not be used across the whole range of parameters or batches.
- Mix the concentrated conjugates, controls and patient samples thoroughly before use. Avoid the build-up of foam.
- The covering films are intended for single use only.
- The packages bear an expiry date. Once 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 the patient samples and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells.
- 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 microtitre wells have been coated with inactivated whole cell lysates, bacterial or viral antigens.
- After the addition of patient or control specimens, the microtitre wells must be considered to be potentially infectious and handled accordingly.
- Donors' blood, in which no antibodies against HIV 1/2, HCV and hepatitis Bs antigen have been detected, is used for the manufac-



ture of the control material. The control material must be treated with the same care as a patient sample, as infection cannot be excluded with total certainty.

- Suitable disposable gloves must be worn throughout the entire test procedure.
- The conjugates contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion, chloroazetamide 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.
- Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
- All fluids to be disposed of must be collected. All collecting containers must contain suitable disinfectants for the 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 stated by the manufacturer must be observed.
- Only use microtitre wells once.
- 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 follow them carefully. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and Preparation

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, haemolytic, lipemic or turbid samples is not recommended.
Caution!
If the tests are not conducted immediately, the sample can be stored for up to 2 weeks at +2 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.

7.2 Preparation of solutions

The test reagents are sufficient for 96 test runs. The following quantity specifications relate to the processing of a single microtitre plate strip with 8 wells respectively. While using several microtitre plate strips, the specified quantities must be simultaneously multiplied with the number of used microtitre plate strips respectively. The device-specific dead volume must be taken into account. The dilution buffers, substrate and stop solution are ready to use.

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

The wash buffer concentrate is diluted **1 + 9** with H_2O (deionised water). 5 ml concentrate is mixed with 45 ml H_2O (deionised water) per microtitre plate strip with 8 wells. The ready-to-use wash buffer can be stored for four weeks at +2 ºC - +8 ºC or for one week at room temperature.

7.2.2 *Preparation of conjugate solution*

For each microtitre plate strip with 8 wells, 1 ml of dilution buffer and 10 µl of anti-human IgG peroxidase conjugate (red cap) or IgM peroxidase conjugate (green cap) are transferred to a clean container and mixed well (dilution **1 + 100**). The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.

8 Test procedure

No.	Execution	Note
1	Expose all reagents for at least 30 minutes to 18-25 °C (room temperature) before beginning the test.	Bring the microtitre plate to room temperature **in a sealed bag**, to avoid condensation of water. Following the removal of the required strips, the plate must be resealed in the bag and stored in the refrigerator. Before use, mix the control and patient serums, as well as the concentrated conjugates thoroughly and then centrifuge briefly if possible, so as to collect the fluid at the bottom of the containers.
2 a)	Preparing samples and controls Pipette 10 µl sample and / or control to every **1 ml** dilution buffer and mix well (dilution **1 + 100**).	**The dilution of the samples and controls must always be performed just before carrying out the test.** For each test step, all of the controls must be carried out, diluted just like the patient samples.
2 b)	The IgM samples are pre-treated for rheumatoid factor absorption. Mikrogen recommends processing the IgM samples by measuring their RF absorption with rheumatoid factor absorbent (Siemens Item No.: OUCG15). After pretreatment, the sample is to be diluted as described under 2a for the test preparations. (The IgM controls may not be pretreated with RF absorbent)	The specific detection of IgM antibodies may result in falsely positive test results in the presence of rheumatoid factors, which is why MIKROGEN recommends pre-treating the serums for IgM determination using rheumatoid factor absorbents. The "Recommendations for the performance of the RF absorption test" can be requested from MIKROGEN.
3	Incubation of samples Pipette **100 µl** of diluted sample and/or diluted control into each well and incubate for **1 hour** at **+37 °C**.	Assign at least one value from the negative control, positive control and patient samples. The cutoff control must be assigned twice. Preferably a cutoff control should be included at the beginning of the series and at the end of the series respectively. In manual processing, carefully cover tightly the microtitre plate with unused cover film. Use the incubation chamber at + 37 °C.
4	Washing	It is recommended to carry out this step with a corresponding ELISA wash device. It is mandatory to ensure that the wash buffer is completely removed between the washing steps.
a)	Carefully remove the covering film.	
b)	Completely empty the wells	Suck off or pour out and beat out the contents.
c)	Fill each of the wells with **300 µl** of ready-to-use wash buffer → (8.4b)	Carry out the washing steps 8.4b and 8.4c **four times** in total.
5	Incubation with conjugate Add **100 µl** of diluted conjugate solution (7.2.2) and incubate for **30 minutes** at **+37 °C**.	In manual processing, the microtitre plate is carefully covered tightly with unused cover film.
6	Washing (see 8.4b and 8.4c).	Carry out the washing steps **four times** in total.
7	Substrate reaction Pipette **100 µl** of ready-to-use substrate solution into each well and incubate for **30 minutes** at **room temperature**. The time is calculated from pipetting into the first well.	Masking of the plate is not required. Protect against direct exposure to sunlight.
8	Stopping the reaction Pipette **100 µl** of ready-to-use stop solution into each well.	The substrate solution is not to be removed before adding the stop solution! The same pipetting scheme is to be followed as for the substrate solution.
9	Measurement of the extinction values The extinction values of the single wells are measured in a microtitre plate photometer at 450 nm and the reference wave length 650 nm (620 to 650 nm permitted).	Zero adjustment is done against air. The measurement must be made within 60 minutes of stopping the reaction.

Caution!
Incubation solutions may not flow into other wells. Splashing must be avoided especially when removing and placing the covering film.

9 Results

9.1 Evaluation

Cutoff (limit) = the average is formed from the extinction values of both cutoff controls (at the beginning and at the end of the series).

9.1.1 *Qualitative evaluation*

Grey range	lower range = cutoff upper range = cutoff + 20% (cutoff x 1.2)
Negative	Samples with extinction values **below** the grey range
Borderline	Samples with extinction values **within** the grey range
Positive	Samples with extinction values **above** the grey range

9.1.2 *Quantitative evaluation*

The corresponding antibody activity in **units per ml** is assigned to the extinction values using a formula.

U/ml sample	(Extinction sample / extinction cutoff) x 20
Grey range	lower range = 20 U/ml upper range = 24 U/ml
Negative	U/ml sample < 20
Borderline	20 ≤ U/ml sample ≤ 24
Positive	U/ml sample > 24

Samples with a borderline test result should be retested. If the results are still borderline after the second test, a further sample should be taken and tested after some time.

9.2 Validation - Quality Control

The test can be evaluated under the following conditions:

- The single extinction values of the double analysis of the cutoff control do not deviate by more than 20 % from their average.
- Negative control extinction value ≤ 0.150
- Cut-off control extinction value - Negative control extinction value ≥ 0.050 (E_{Cutoff} - $E_{neg.\ controls}$ ≥ 0.050)
- Positive control extinction value - Cutoff control extinction value ≥ 0.300 ($E_{pos.\ contr.}$ - E_{Cutoff} ≥ 0.300)

10 Limitations of the method - restrictions

- Serological test results must always be seen in the context of the clinical picture of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- A negative *recom*Well Parvovirus B19 test result cannot exclude an infection with the human Parvovirus B19. In the event of a clinical suspicion concerning an infection with the Parovirus B19 and negative serological findings, a further sample should be taken and tested after two weeks. In such cases, it is recommended to perform a virus test with PCR as well. False negative results may occur when the serum samples are taken very soon after infection has occurred.
- We generally recommend checking positive and borderline ELISA results by conducting a confirmation test.

11 Test performance

11.1 Sensitivity and specificity

	***recom*Well Parvovirus B19 IgM** % (n)	***recom*Well Parvovirus B19 IgG** % (n)
sensitivity	**98.3%** (59/60)*	**100%** (295/295)*
Specificity	**99.8%** (430/431)*	**98.7%** (149/151)*

*With rheumatoid (RF) absorption: MIKROGEN recommends using an RF absorbent (RF absorbent by Dade Behring))

Samples from routine practice, samples from pregnant women and samples of children with acute Parovirus B-19 infection were tested to calculate the sensitivity and specificity. The data were compared with another, commercially available ELISA that also works with VP2 particles.

11.2 Relative correlation

The determination of positive and negative correlation was carried out in a comparison with a commercially available confirmation test (*recom*Line Parvovirus B19).

	***recom*Well Parvovirus B19 IgM % (n)**	***recom*Well Parvovirus B19 IgG % (n)**
Positive correlation[1]	98.0 % (49/50)	99.1 % (114/115)
Negative correlation[1]	97.9% (93/95)	100 % (33/33)

[1]Samples with a borderline serological result were not incorporated into the study.

11.3 Sero-prevalence

	***recom*Well Parvovirus B19 IgM seropositive**	***recom*Well Parvovirus B19 IgG seropositive**
Seroprevalence for blood donors (n=200 plasma samples, n=100 serum samples)	0.3% (1/300)*	81.3% (244/300)

* The positive IgM result was not confirmed by a confirmation test (*recom*Blot Parvovirus B19 IgM). The evaluation of the *recom*Well Parvovirus B19 IgM was performed using RF-absorption (RF-absorbent from Dade Behring).

11.4 Analytical sensitivity

	***recom*Well Parvovirus B19 IgG Result and/or extinction value**
WHO standard* ≈ 2 IU/ml	Result: borderline (20 ≤ U/ml sample ≤ 24)
WHO standard* ≈ 3 IU/ml	Result: borderline (U/ml sample > 24)
WHO standard* 50 IU/ml	Extinction: 2.5
WHO standard* 100 IU/ml	Extinction: 2.8

* WHO standard (NIBSC code 93/724) was obtained from the National Institute for Biological Standards and Control (Hertfordshire, UK).

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 potential interfering factors have shown that the performance of the test is not affected by anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia of the sample.

b) Cross-reactions: Potential interference from antibodies against EBV, as well as from other conditions that are due to atypical behaviour of the immune system (anti-nuclear auto-antibodies, rheumatoid factor [positive after RF absorption) can be practically ruled out.

11.6 Precision

	***recom*Well Parvovirus B19 IgM VK**	***recom*Well Parvovirus B19 IgG VK**
Intra-assay variance*	5.7%,	5.2%
Inter-assay variance**	< 16%	< 14.4%

*A patient sample was measured in 96 wells of a micro-titration plate. The variation coefficient (VC) for the extinct values was calculated.
** Patient samples with different reactivity were examined on 6 or 5 different days within a period of 35 or 30 days in the IgG or IgM. Two different batches were used.

12 Literature

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We will gladly send you further literature on the diagnosis of Parvovirus B19 on request.

13 Explanation of symbols

Symbol	Meaning
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 number
	Do not freeze
REF	Order number
	Best before Expiry date
x°C – y°C	Store at x°C to y°C

14 Manufacturer and version information

***recom*Well Parvovirus B19 IgG** ***recom*Well Parvovirus B19 IgM**	Item no. **4404** Item no. **4405**
Instructions for use valid from	GAREPA009EN February 2012

MIKROGEN GmbH
Floriansbogen 2-4
82061 Neuried
Germany
Tel. +49 89 54801-0
Fax +49 89 54801-100
E-mail mikrogen@mikrogen.de
Internet www.mikrogen.de

QM system certified by:

Zertifiziertes QM-System mdc ISO 13485

CE