

IVD

Instructions for use (English)

1 Purpose

The *recomWell FSME / TBE Virus* is a qualitative and quantitative *in vitro* test for the reliable identification of IgG or IgM antibodies to the tick-borne encephalitis (FSME) virus in human serum, plasma or CSF. The *recomWell FSME / TBE Virus* is an indirect sandwich ELISA method.

2 Intended use

The *recomWell FSME / TBE Virus* is indicated for use in the following cases:

- Proof of seroconversion following vaccination (as per the vaccine maker's recommendations)
- Proof of suspected or manifest FSME virus infection (differential diagnosis for Borreliosis following tick bite or to rule out other conditions of the CNS)
- Monitoring following FSME infection
- Proof of intrathecal FSME virus-specific antibodies in the CSF

3 Test principle

When using the *recomWell FSME / TBE Virus*, microtitre plates are coated with inactivated FSME virus antigen.

1. Diluted serum or plasma samples are incubated in the wells, wherein antibodies bind 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 with a staining reaction catalysed by the peroxidase. If an antigen/antibody-reaction occurs, the chromogen substrate solution colours proportionally to the quantity of the bound anti-FSME virus IgG or IgM antibodies. The intensity of the colour development can be measured using a photometer. The concentration of the anti-FSME virus antibody in the sample can be deduced in this way.

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 Oxypryion (0.1%)
DILUBUF	125 ml Dilution buffer (ready-to-use) Contains protein, detergent and blue dye. Preservative: MIT (0.01%) and Oxypryion (0.1%)
SUBS TMB	12 ml Chromogenic substrate tetramethylbenzidine (TMB, ready-to-use)
SOLN STOP	12 ml stop solution 24.9% phosphoric acid (H₃PO₄) (ready-to-use)
INSTRU	1 Instructions for use
EVALFORM	1 Evaluation form
TAPE	2 pieces of covering film

recomWell FSME / TBE Virus IgG also contains:

MTP	12X8 wells Microtitre plate (cap strip marked red) coated with inactivated FSME virus antigen in the self-sealing vacuum bag
CONTROL ± IgG	150 µl positive control (violet cap) contains MIT (0.1%) and Oxypryion (0.1%)
CONTROL ± IgG	150 µl cutoff control (yellow cap) contains MIT (0.1%) and Oxypryion (0.1%)
CONTROL - IgG	150 µl negative control (white cap) contains MIT (0.1%) and Oxypryion (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%)

Calibrators (see 9.1.2) are available upon request.

Calibrators 1-5 Art. No. 20101	5 x 350 µl CAL 1, CAL 2, CAL 3, CAL 4, CAL 5 Human sera with stabilisers and preservatives (manufacturer: PROGEN Biotechnik GmbH, Heidelberg)
--	--

recomWell FSME / TBE Virus IgM also contains

MTP	12X8 wells Microtitre plate (cap strip marked green) coated with inactivated FSME virus antigen in the self-sealing vacuum bag
CONTROL + IgM	150 µl positive control (black cap) contains MIT (0.1%) and Oxypryion (0.1%)
CONTROL ± IgM	150 µl cutoff control (colourless cap) contains MIT (0.1%) and Oxypryion (0.1%)
CONTROL - IgM	150 µl negative control (white cap) contains MIT (0.1%) and Oxypryion (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

5 Shelf life and handling

- Store reagents at +2 to +8 °C before and after use, **do not freeze**.
- Expose 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 *recomWell* test can be used across the whole range of parameters and batches. The shelf life of these components should 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 sera thoroughly before use. Avoid a build up of foam.
- The covering films are intended for single use only.
- The packages bear an expiration date. After this has been reached, no guarantee of quality can be offered.
- Protect the 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 to the product or the regulations for use by the user, the application may lie outside the purpose indicated 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; 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 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 treated accordingly.
- Donors' blood, in which no antibodies against HIV 1/2, HCV and HBs antigen have been detected, is used for the manufacture of the control specimen. The product must be treated with the same care as with 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), oxyprion, 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.
- ♣ Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
- ♣ All fluids must be collected that are intended for disposal. 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 the entire instructions for use before carrying out the test and be sure to 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 (EDTA, citrate, heparin, CPD), which must be separated as soon as possible from the blood clot after blood sampling so as to avoid a haemolysis. CSF can also be used. Avoid microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation. The use of heat-inactivated, icteric, haemolytic, lipaemic 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 to +8 °C. Prolonged storage of the samples is possible by keeping them at -20 °C or less. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results.

7.2 Preparation of solutions

The detection reagents are sufficient for 96 IgG and/or IgM analyses. 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 considered. Dilution buffer, 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 H₂O (deionised water). 5 ml concentrate is mixed with 45 ml H₂O (deionised water) per microtitre plate strip with 8 wells. The ready-to-use wash buffer can be stored for four weeks at +2°C - +8 °C 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	<u>Preparing samples and controls</u> Pipette 10 µl of sample and/or control to every 1 ml of 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. The specific detection of IgM antibodies can lead to false positive test results in the presence of rheumatoid factors. We recommend pre-treating sera used for IgM detection with rheumatoid factor absorbent. In this instance too, the sample dilution of 1 + 100 must be adhered to. The IgM control sera must not be treated with the RF absorbent! For each test step, all of the controls must be carried out, diluted just like the patient samples.
3	<u>Incubation of samples</u> 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 the case of manual processing, carefully remove the microtitre plate with the unused cover film. Use the incubation chamber at + 37 °C.
4	<u>Washing</u> a) Carefully remove the covering film. b) Completely empty the wells c) Fill each of the wells with 300 µl of ready-to-use wash buffer → (8.4b)	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. Suck off or pour out and beat out the contents. Carry out the washing steps 8.4b and 8.4c four times in total.
5	<u>Incubation with conjugate</u> Add 100 µl of diluted conjugate solution (7.2.2) and incubate for 30 minutes at +37 °C .	With manual processing, the microtitre plate is to be removed carefully with the unused cover film.
6	<u>Washing</u> (see 8.4b and 8.4c).	Carry out the washing steps four times in total.
7	<u>Substrate reaction</u> 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 <u>not</u> required. Protect against direct exposure to sunlight.
8	<u>Stopping the reaction</u> Pipette 100 µl of ready-to-use stop solution <u>into</u> 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	<u>Measurement of the extinction values</u> 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.		

8.1 Cerebrospinal fluid / Serum analytics

A separate manual, patient protocol and evaluation software are available for the detection of human IgG and IgM antibodies intrathecally formed in the cerebrospinal fluid (cerebrospinal fluid / serum pairs). You can request the current versions from Mikrogen (+ 49 89 54801-0) or download at (www.mikrogen.de → Downloads).

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. Where there is a clinical suspicion of FSME and the serum findings are negative or inconclusive, a further sample should be taken for testing after 7 to 10 days.

We also recommend carrying out a further check on borderline IgG or IgM results.

There is currently no international reference serum for the quantification of anti-FSME virus antibodies. ELISA results cannot therefore be expressed in international units. One method of quantification is "Vienna Units" (VIEU/ml, after Prof. Kunz, Vienna). To determine the number of VIEU/ml, a calibrator set (5-point calibration) can be obtained from Mikrogen: Art. No. 20101.

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 ($E_{\text{Cutoff}} - E_{\text{neg. contr.}} \geq 0.050$)
- Positive control extinction value - Cutoff control extinction value ≥ 0.300 ($E_{\text{pos. contr.}} - E_{\text{Cutoff}} \geq 0.300$)

10 Limitations of the method - restrictions

- Serological test results must always be seen in the context of the clinical picture of the patient. The therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- A negative *recomWell* FSME / TBE Virus test result cannot exclude infection with the FSME virus. If there is clinical suspicion of infection with the FSME / TBE virus, e.g. following a tick bite, a further sample should be taken for testing after 7 to 10 days.
- A positive *recomWell* FSME / TBE Virus test result does not always mean that there is an active disease process in progress, since this may also be due, as a result of the close relationship with other members of the Flaviviridae family, to an infection or vaccination with yellow fever, Dengue, Japanese encephalitis, West Nile virus and other pathogens.
- Serological test results must always be seen in the context of the clinical picture. Consequently, if FSME / TBE virus antibodies are detected for the first time and the patient has clear clinical symptoms, an FSME / TBE virus infection is likely if other causes, such as Lyme Borreliosis, have been ruled out. Consequently, a test for anti-Borrelia antibodies is recommended in the event of a (suspected) tick bite.
- To detect intrathecal antibodies, serum CSF pairs need to be tested in parallel in the *recomWell* FSME / TBE Virus. Instructions on how to perform this test can be obtained from MIKROGEN

(FSME / TBE Virus CSF diagnostics). An Excel programme can be requested from Mikrogen for facilitating the calculations for CSF diagnostics (after Reiber) (see 0).

- Proof of seroconversion following vaccination is provided by detecting specific IgG antibodies. In this case, the recommendations of the vaccine manufacturer should be followed in regards to the time of the test. In some cases, the vaccination can also cause the parallel development of IgM antibodies over the course of several months, but mostly at a low titre.

11 Test performance

11.1 Diagnostic sensitivity

<i>recomWell</i> FSME / TBE Virus IgG % (n)	<i>recomWell</i> FSME / TBE Virus IgM % (n)
100% (155/155)	100% (133/133)

Clinically-defined samples that were consistently reactive (i.e. positive or borderline) in five commercially - available comparison tests (ELISA), origin: State Office for Health and Food Safety, Oberschleissheim.

11.2 Diagnostic specificity

<i>recomWell</i> FSME / TBE Virus IgG % (n)	<i>recomWell</i> FSME / TBE Virus IgM % (n)
100% (124/124)	100% (196/196)

Samples that yielded a consistently negative reaction in two commercially-available comparison tests (ELISA), origin: Bavarian Red Cross.

11.3 Seroprevalence

<i>recomWell</i> FSME / TBE Virus IgG		<i>recomWell</i> FSME / TBE Virus IgM	
Seropositive % (n)	Seronegative % (n)	Seropositive % (n)	Seronegative % (n)
33.0% (66/200)	67.0% (134/200)	0.0% (0/200)	100.0% (200/200)

Seroprevalence for the FSME / TBE virus among tested blood donors was determined using two comparison tests (ELISA) for IgG at 32.5% and 36.5% and for IgM at 1.0% and 1.5%. Origin of samples: Bavarian Red Cross. Seroprevalence is greatly dependent on the origin of the samples. The IgG levels can also relate to vaccination titres.

11.4 Precision

	IgG	IgM
Intra-assay	VK = 5.6%	VK = 9%
Inter-assay	VK < 13%	VK < 6%

11.5 Analytical specificity

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 (e.g. anticoagulants, haemolysis, effects of the sample treatment) or cross reactions with potentially interfering antibodies.

a) **Interferences:** Control studies on potential interfering antibodies have shown that the performance of the test is not affected by anticoagulants (citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia.

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) can be ruled out. Cross-reactions with antibodies against other members of the Flaviviridae family cannot be ruled out.






12 Literature

1. Holzmann H., Kundi M., Stiasny K., Clement J., McKenna P., Kunz C., Heinz F.X. (1996) Correlation between ELISA, Hemagglutination Inhibition, and Neutralization Tests after Vaccination against Tick-borne Encephalitis. *Journal of Medical Virology* 48, 102 – 107
2. Holzmann H. (2003) Diagnosis of tick-borne encephalitis. *Vaccine* 21 S1/36 – S1/40
3. Kaiser R. (2008) Tick-borne encephalitis. *Infectious Disease Clinics of North America* 22, 561 – 575
4. Kaiser R. (2005) Neuroborreliose und Frühsommer-Meningoenzephalitis – Gemeinsamkeiten und Unterschiede. *Fortschr. Neurol. Psychiat.* 73, 750 – 764
5. Mansfield K.L., Johnson N., Phipps L.P., Stephenson J.R., Fooks A.R., Solomon T. (2009) Tick-borne encephalitis virus – a review of an emerging zoonosis. *Journal of General Virology* 90, 1781 – 1794
6. Mantke O.D., Schädler R., Noedrig M. (2008) A survey on cases of tick-borne encephalitis in European countries. *Eurosurveillance* 13, Issues 4-6
7. Niedrig M., Avsic T., Aberle S.W., Ferenczi E., Labuda M., Rozentale B., Mantke O.D. (2007) Quality control assessment for the serological diagnosis of tick borne encephalitis virus infection. *Journal of Clinical Virology* 38, 260 – 264

8. Niedrig M., Vaisviliene D., Teichmann A., Klockmann U., Biel S.S. (2001) Comparison of six different commercial IgG-ELISA kits for the detection of TBEV-antibodies. *Journal of Clinical Virology* 20, 179 – 182
9. Paulke-Korinek M., Rendi-Wagner P., Kundi M., Laaber B., Wiedermann U., Kollaritsch H. (2009) Booster vaccinations against tick-borne encephalitis: 6 years follow-up indicates long-term protection. *Vaccine* 27, 7027 – 7030
10. Stiansny K., Holzmann H., Heinz F.X. (2009) Characteristics of antibody responses in tick-borne encephalitis vaccination breakthroughs. *Vaccine* 27, 7021 – 7026
11. Süss J., Kahl O., Aspöck H., Hartelt K., Vaheiri A., Oehme R., Hasle G., Dautel H., Kunz C., Kupreviciene N., Randolph S., Zimmermann H.-P., Atkinson B., Dobler G., Kutsa K., Heinz F.X., Steffen R. (2010) Tick-borne encephalitis in the age of general mobility. *Wiener Medizinische Wochenschrift* 160/3-4, 94 – 100

We would be glad to send you further literature on FSME diagnostics on 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 number
	Do not freeze
 REF 	Order number
	Best before Expiry date
	Store at x°C to y°C

14 Manufacturer and version information

recomWell FSME / TBE Virus IgG	Item. No. 6504
recomWell FSME / TBE Virus IgM	Item. No. 6505
Instructions for use valid from	GAREFS002EN March 2011
MIKROGEN molekularbiologische Entwicklungs-GmbH Floriansbogen 2-4 82061 Neuried Tel. +49 89 54801-0 Fax +49 89 54801-100 E-mail mikrogen@mikrogen.de Internet www.mikrogen.de	
QM system certified by:	