*recom*Well HEV IgG
*recom*Well HEV IgM

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Well HEV IgG, IgM is a qualitative and/or quantitative in vitro test for the detection and reliable identification of IgG or IgM antibodies to Hepatitis E Virus in human serum or plasma. The *recom*Well HEV IgG, IgM is a screening test based on the principle of an indirect sandwich ELISA.

2 Intended use

The *recom*Well HEV IgG, IgM detects IgG and IgM antibodies against Hepatitis E Virus.
The clinical picture of a human hepatitis E infection ranges from inapparent to fulminant. In general the hepatitis E infection is an acute self-limiting viral hepatitis infection similar to the hepatitis A infection. However, recently a few clinical cases were described on suspicion of being chronical. A potential parenteral transmission via blood or transplants beside the known fecal-oral route of transmission is propable. During the last years an increased number of hepatitis E infections without the known travel history (India, Pakistan, Mexico, SE-Asia) were diagnosed as autochthonous hepatitis E infections in the northern industrialized countries. At least four different genotypes exist of the human pathogen hepatitis E virus worldwide. The human HEV genotype 3 shows a high homology to the swine hepatitis E virus. The prevalence of HEV infections in pigs is up to 80% so a food-borne zoonotic disease is being discussed.

3 Test principle

Highly purified recombinant HEV-ORF2 (genotype 1 and genotyp 3) virus antigens are fixed to the wells of a microplate.

1. Diluted serum or plasma samples are incubated in the wells, in which 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, 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-catalyzed colour reaction. Where an antigen-antibody reaction has taken place, the colour development of the chromogenic substrate is proportionate to the quantity of bound HEV IgG, IgM antibodies. The concentration of colour development can be measured using a photometer, so as to establish the concentration of anti-HEV IgG, IgM 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-to-use**)
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form
TAPE	**2 pieces** of covering film

***recom*Well HEV IgG** also contains:

MTP	**12x8 wells** microplate (section marked in **red**), coated with recombinant HEV antigens in a vacuum pressure-sealed bag
CONTROL + IgG	**450 µl** positive control (**violet** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgG	**450 µl** cutoff control (**yellow** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgG	**450 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgG	**500 µl** anti-human IgG conjugate (**101-times concentrated**, **red** cap) contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

***recom*Well HEV IgM** also contains:

MTP	**12x8 wells** microplate (section marked in **green**), coated with recombinant HEV antigens in a vacuum pressure-sealed bag
CONTROL + IgM	**450 µl** positive control (**black** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgM	**450 µl** cutoff control (**colourless** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgM	**450 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgM	**500 µl** anti-human IgM 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°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 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, control specimens and patient samples well before use. Avoid build-up of foam.
- All MIKROGEN microtiter plates are equipped with Break-a-part-bars.
- The covering films are intended for single use only.
- All packages are marked with an expiry date, after which quality can no longer be guaranteed.
- 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 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 potentially infectious and treated accordingly.
- For the production of control specimens, blood from donors is used which does not contain antibodies to HIV 1/2, HCV and HBs antigen. The product must be treated with the same care as for a patient sample, as an infection cannot be excluded with 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.
- Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
- All fluids to be disposed 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 carefully follow them. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and Preparation

7.1 Samples

The samples can be serum or plasma (EDTA, citrate, heparin, CPD) and must be separated from the blood clot as soon as possible after blood 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 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 less. 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

Contains sufficient reagents for 96 tests. 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. 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 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 +2ºC to +8 ºC or a week at room temperature.

7.2.2 *Preparation of conjugate solution*
For each microplate strip with 8 wells, 1 ml of dilution buffer and 10 µl of anti-human IgG-peroxidase conjugate (red cap) or 10 µl of anti-human IgM-peroxidase conjugate (green cap) are transferred to a clean container and mixed well (dilution **1 + 100**). The conjugate solution must be prepared <u>just before use</u>. 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°C to +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, thoroughly mix the control specimens and patient samples, as well as the concentrated conjugates and then centrifuge briefly, where possible, in order 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** dilution buffer and mix well (dilution **1 + 100**).	**Samples and control specimens must always be diluted immediately prior to carrying out the test.** 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 manual processing, carefully cover tightly the microtitre plate with unused cover film. Use the incubation chamber at +37°C.
4 a) b) c)	<u>Washing</u> Carefully remove the covering film. Completely empty the wells 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**.	In manual processing, the microtitre plate is carefully covered tightly with 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.	It is <u>not</u> required to tightly cover the plate. 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.**		

9 Results

9.1 Evaluation

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

9.1.1 *Qualitative evaluation*

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

9.1.2 *Quantitative evaluation*
The corresponding antibody activity in **units per ml** is assigned to the extinction values using a formula. The measurement units U/ml are arbitrary units, which do not allow conclusions concerning (international) reference values.

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

Samples showing a borderline test result should be retested. If they are still found to be borderline by the second test, it is recommended to sample again after a certain period of time and test again.

The linearity of the test was determined during the evaluation within the following range:
20 U/ml to 125 U/ml (R^2 = 0.95)

In case of an extinction ≥ 3.0 or a measuring value above the linear range, the result should either be given as > 125 U/ml, or the sample may be diluted and measured again. We recommend to start with a final sample dilution of 1:500 and if necessary further subsequent dilution steps.

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.
- Extinction value – negative control ≤ 0.150
- Cutoff contr. extinction value - Negative contr. extinction value ≥ 0.050 (E_{Cutoff} - $E_{neg.\ contr.}$ ≥ 0,050)
- Positive contr. extinction value - Cutoff contr. extinction value ≥ 0.300 ($E_{pos.\ contr.}$ - E_{Cutoff} ≥ 0.300)

These checks are used to validate the test results as per the "Validation Quality Control" chapter. The reproducibility of results can be improved by determining the specific antibodies relative to the cut-off check in U/ml, as the fluctuations from the performance of the test are also included. In validating the test, the positive and negative checks do not need to be evaluated. If necessary, however, they can be carried out for internal quality control purposes. In this case, the results should lie within the target value range given in the analysis certificate or on the label.

9.3 Interpretation table

IgG	IgM	Test interpretation
negative	negative	No indication of HEV infection. If clinical suspicion persists, a follow-up should be performed after about 1-2 weeks.
negative	positive	IgM antibodies against HEV in evidence. Possible early stage of HEV infection. Direct pathogen detection or follow-up after about 1-2 weeks recommended.
positive	positive	Indication of an acute HEV infection.
positive	negative	IgG antibodies against HEV present. There may have been a recent infection or a long past infection.

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.
- Where the test results are unclear or inconclusive, it is recommended to repeat testing over the course of the infection. In addition to the laboratory results, clinical findings and associated history must be taken into account for each case in order to establish the diagnosis of HEV infection and the classification of the various stages.
- A positive result with *recom*Well HEV IgG indicates a previous or an active primary infection. An additional IgM test result is necessary to complete the serological diagnosis (see table 9.3).
- An isolated positive test result with *recom*Well HEV IgM may point to an acute HEV infection. Further sampling and testing should be carried out after two to three weeks. Usually an IgG titer appears with a short delay.
- When results with *recom*Well HEV IgG and/or IgM are positive or borderline, we recommend to follow-up with a confirmatory test (e.g. *recom*Line HEV IgG/IgM).
- Cross-reactions with antibodies, produced by infection with other viruses (e.g. Hepatitis A, B, C, cytomegalovirus), can largely be excluded due to the use of selected recombinant HEV antigens.

11 Test performance
11.1 Diagnostic sensitivity and specificity

*recom*Well HEV IgG	HEV-seronegative* n = 134	acute HEV infection n =89
Diagnostic sensitivity	-	98.9%
Diagnostic specificity	98.5%	-

* Blood donor sera have been concordantly evaluated as negatives in another HEV ELISA IgG and in *recom*Line HEV IgG.

*recom*Well HEV IgM	HEV-seronegative** n = 359	acute HEV Infection n =89
Diagnostic sensitivity	-	98.9%
Diagnostic specificity	98.6%	-

** 159 sera from patients with clinical suspicion of non-E hepatitis; serologically defined by a *recom*Well ELISA, and by another ELISA, and/or by a *recom*Line assay; positive for either HBs-IgM-, HAV-IgM-, CMV-IgM- Parvo-IgM antibodies, HCV-IgG antibodies; and 200 negative blood donors.

11.2 Seroprevalence of Anti-HEV-antibodies in blood donors

n = 200	*recom*Well HEV IgG	*recom*Well HEV IgM
positive	60	2
borderline	6	0
negative	134	198
seroprevalence	33%	1%

11.3 Analytical specificity
The analytical specificity is defined as the capacity of the test to determine the analytes accurately 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 showed that anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia of the sample have no impact upon the efficiency of the test.
b) Cross reactions: The potential interference of antibodies with other organisms that can produce similar clinical symptoms as in an HEV infection (e.g. hepatitis A-C-, EBV, cytomegalovirus, Parvovirus B19) was investigated in control studies. Conditions were also tested that display an atypical activity of the immune system (e.g. antinuclear autoimmune antibodies, pregnancy, rheumatoid factor). No crossreactivities can be verified. Exception: In the presence of an active EBV-infection sometimes HEV-IgM-antibodies can be detected.

11.4 Precision

	*recom*Well HEV IgG	*recom*Well HEV IgM
Intra-assay-variance*	CoV < 8.6%	CoV < 7.9%
Inter-assay-variance**	CoV < 5.2%	CoV < 7.6%

*Three positive or borderline patient samples were tested in 10 to 12 cavities each in a diagonal pattern on a microplate. The coefficient of variation (CoV) was calculated for the U/ml of the samples.
**Three positive or borderline samples of different levels of extinction were examined three times on three different days in quaduplicate. The coefficient of variation (CoV) was calculated for the U/ml of the samples.

12 Literature
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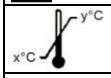
Further Information on hepatitis E Virus diagnostics is available on request.

13 Explanation of symbols

	Content is sufficient for <n> applications Number of applications
WASHBUF 10 X	Wash Buffer (ten times concentration)
DILUBUF	Dilution Buffer
SUBS TMB	Chromogenic substrate Tetramethylbenzidin
SOLN STOP	Stop solution
TAPE	Covering films
MTP	Microtitre plate
CONTROL + IgG	Positive controls IgG
CONTROL ± IgG	Cut-off controls IgG
CONTROL - IgG	Negative controls IgG
CONJ IgG	Anti-human IgG conjugate
CONTROL + IgM	Positive controls IgM
CONTROL ± IgM	Cut-off controls IgM
CONTROL - IgM	Negative controls IgM
CONJ IgM	Anti-human IgM conjugate
TVALUE	Target and / or target range in U/ml
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
IVD	In vitro diagnostic 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*Well HEV IgG** ***recom*Well HEV IgM**	Item No. **5004** Item No. **5005**
Instructions for use valid from	GAREHE013EN 2023-04

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