TBE/FSME

IgG – ELISA

Enzyme immunoassay for the quantitative determination of IgG-class antibodies against TBE Virus in human serum or plasma

For laboratory research only.

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Product Number: 40-521-475128 (96 Determinations)
1. INTRODUCTION

Tick-borne encephalitis (TBE) virus is a flavivirus of the family Togaviridae. It is an enveloped single-stranded RNA virus with cubic icosahedral symmetry and ranges in size from 20-80nm in diameter.

On the European continent only two antigenic subtypes exist which show only little differences in their structural proteins. TBE virus is mainly transmitted by ticks. The degree of contamination of ticks (and thus humans) in central Europe increases from west to east, and anybody may be affected. Specific antibody development yields a life-long immunity.

TBE is the most important tick-transmitted disease of man –beside Lyme disease, which is caused by the spirochete Borrelia burgdorferi. The clinical course of the disease depends on the immune status of the infected persons. A high virus production in the primary infected tissues is required for the passage of the blood-brain barrier and the resulting severe manifestations in the central nervous system.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Symptoms</th>
<th>Mechanism of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBE Virus</td>
<td>Tick-borne encephalitis</td>
<td>Mild, influenza-type illness; fever; severe headache and neck rigidity with transient paralysis of the limbs, shoulders or, less commonly, the respiratory musculature; nausea</td>
<td>Transmission mainly by tick bites (Ixodes ricinus, western subtype; Ixodes persulcatus, eastern subtype), but also through ingestion of infected (non-pasteurized) milk. No transmission from one person to another. Incubation period: 7-14 days</td>
</tr>
<tr>
<td>- CEE (Central European Encephalitis)</td>
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<td></td>
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<tr>
<td>- RSSE (Russian Spring Summer Encephalitis)</td>
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</tbody>
</table>

Of the two subtypes, RSSE causes the more severe infection, having an incidence of mortality of up to 25% in some outbreaks, whereas mortality in CEE seldom exceeds 5%. An inactivated TBE virus vaccine is available in Europe and Russia. The presence of virus resp. infection may be identified by:
- Haemagglutination-Inhibition, Complement Fixation
- Detection of specific antibodies by CIE, ELISA

2. INTENDED USE

The GenWay TBE/FSME IgG-ELISA is intended for the quantitative determination of IgG class antibodies against TBE virus in human serum or plasma (citrate).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of IgG-class antibodies against TBE Virus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiterstrip wells are precoated with TBE Virus antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured TBE Virus specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of TBE Virus specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied
- **TBE/FSME Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with TBE Virus antigen; in resealable aluminium foil.
- **IgG Sample Diluent***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2; white cap.
- **TBE/FSME anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labelled antibodies to human IgG; coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3’,5,5’-tetramethylbenzidine (TMB); ready to use; yellow cap.
TBE/FSME IgG Standards**: 5 vials, each containing 2ml; coloured yellow; ready to use:
- Standard A: 0 NTU/ml; blue cap
- Standard B: 50 NTU/ml; green cap
- Standard C: 130 NTU/ml; yellow cap
- Standard D: 200 NTU/ml; red cap
- Standard E: 300 NTU/ml; white cap

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied
- 1 Strip holder
- 1 Cover foil
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE
The reagents are stable up to the expiry date stated on the label when stored at 2…8 °C.

6. REAGENT PREPARATION
It is very important to bring all reagents, samples and controls to room temperature (20…25°C) before starting the test run!

6.1. Coated Snap-off Strips
The ready to use breakapart snap-off strips are coated with TBE Virus antigen. Store at 2…8°C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date.

6.2. TBE/FSME anti-IgG Conjugate
The bottle contains 20 ml conjugate with the components anti-human IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

6.3. Standards
The vials labelled with Standard A, B, C D and E contain a ready to use standard solution. The concentration of the standards are:
- Standard A: 0 NTU/ml
- Standard B: 50 NTU/ml
- Standard C: 130 NTU/ml
- Standard D: 200 NTU/ml
- Standard E: 300 NTU/ml

The solutions have to be stored at 2…8°C and contain 0.1% Kathon. After first opening stability until expiry date when stored at 2…8°C.

6.4. IgG Sample Diluent
The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

6.5. Washing Solution (20xconc.)
The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. Crystals in the solution disappear by warming up to 37 °C in a water bath. After first opening the concentrate is stable until the expiry date.
6.6. TMB Substrate Solution
The bottle contains 15 ml of a Tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged. After first opening stability until expiry date when stored at 2...8°C.*

6.7. Stop Solution
The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26), ready to use, store at 2...8°C. After first opening stability until expiry date.

7. SPECIMEN COLLECTION AND PREPARATION
Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.* Heat inactivation of samples is not recommended.

7.1. Sample Dilution
Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:
1 well (e.g. A1) for the substrate blank.
5 wells (e.g. B1, C1, etc.) for Standard A, B, C, D and E.

*It is recommended to determine patient samples in duplicate.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl of each Standard (A, B, C, D and E) and diluted samples into the respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content off the wells and wash each well three times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100µl TBE Virus anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature (20 to 25°C). Do not expose to direct sunlight.**
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature (20 to 25°C) in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.
   *Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.*
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.
8.2. Measurement
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Assay Validation Criteria
In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance < **0.100**.
- **Standard A** in B1: Absorbance < **0.200**
- **Standard B** in C1: Absorbance > **0.200**
- **Standard C** in D1: Absorbance > **0.400**
- **Standard D** in E1: Absorbance > **0.800**
- **Standard E** in F1: Absorbance > **1.200**

Standard A < Standard B < Standard C < Standard D < Standard E

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results
In order to obtain quantitative results in NTU/ml plot the (mean) absorbance values of the 5 Standards A, B, C, D and E on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 50, 130, 200 and 300 NTU/ml) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis). Read results from this standard curve employing the (mean) absorbance values of each patient specimen and control. As a matter of course, all suitable computer programs available can be used for automated result reading and calculation.

9.3. Interpretation of Results
Normal value ranges for this ELISA should be established by each laboratory based on its own patient populations in the geographical areas serviced.

The following values should be considered as a guideline:

**Positive:** > **110** NTU/ml  
**Grey zone (equivocal):** 55 – 110 NTU/ml  
**Negative:** < **55** NTU/ml

9.3.1 Results after vaccination

**TBE IgG negative**  
No seroconversion after vaccination.  
This may be the case after the first vaccination during basic immunisation.  
Patients which show low or no response.

**TBE IgG grey zone**  
This may be a case of seroconversion.  
Continue with basic immunisation or booster.  
Repeat test within 2–4 weeks.  
A non-specific reaction is not excluded.

**TBE IgG positive**  
This is a case of seroconversion.  
Check anamnestic data and if necessary complete basic immunization or give a booster.
9.3.2. Results after TBE infection

When interpreting the results the anamnesis data and the clinical symptoms have to be taken into account as well as the serological data.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TBE IgG/IgM</td>
<td>negative</td>
<td>No infection with the TBE Virus.</td>
</tr>
<tr>
<td>TBE IgG</td>
<td>positive</td>
<td>Latent immunisation or the infection occurred weeks or months before.</td>
</tr>
<tr>
<td>TBE IgM</td>
<td>negative</td>
<td>A TBE Virus infection is possible. In the early phase the TBE IgG can be negative or grey zone. Repeat test within 7-10 days to monitor the levels of the antibodies.</td>
</tr>
<tr>
<td>TBE IgG</td>
<td>positive</td>
<td>A TBE Virus infection is most probable if no vaccination was performed.</td>
</tr>
<tr>
<td>TBE IgM</td>
<td>positive</td>
<td>It is recommended to verify this result with another test system.</td>
</tr>
<tr>
<td>TBE IgG</td>
<td>grey zone</td>
<td>A new blood specimen has to be tested within 7-10 days to monitor the levels of the antibodies.</td>
</tr>
<tr>
<td>TBE IgM</td>
<td>grey zone</td>
<td></td>
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</table>

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

<table>
<thead>
<tr>
<th>Interassay</th>
<th>n</th>
<th>Mean value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard B</td>
<td>14</td>
<td>0.31</td>
<td>7.35</td>
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<tr>
<td>Standard C</td>
<td>16</td>
<td>0.92</td>
<td>8.3</td>
</tr>
<tr>
<td>Standard D</td>
<td>15</td>
<td>1.37</td>
<td>7.8</td>
</tr>
<tr>
<td>Standard E</td>
<td>16</td>
<td>2.28</td>
<td>6.7</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Intraassay</th>
<th>n</th>
<th>Mean value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard B</td>
<td>7</td>
<td>0.32</td>
<td>6.8</td>
</tr>
<tr>
<td>Standard C</td>
<td>8</td>
<td>0.98</td>
<td>5.7</td>
</tr>
<tr>
<td>Standard D</td>
<td>8</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Standard E</td>
<td>8</td>
<td>2.15</td>
<td>4.2</td>
</tr>
</tbody>
</table>

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is >98%.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is >98%.

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

11. LIMITATIONS OF THE PROCEDURE

Cross reactivity with antibodies of other flaviviruses, for example Dengue Virus, Yellow fever, Japanese encephalitis viruses, cannot be excluded.

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromized patients and newborns serological data only have restricted value.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
• All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
• Do not interchange reagents or strips of different production lots.
• No reagents of other manufacturers should be used along with reagents of this test kit.
• Do not use reagents after expiry date stated on the label.
• Use only clean pipette tips, dispensers, and lab ware.
• Do not interchange screw caps of reagent vials to avoid cross-contamination.
• Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
• After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
• To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
• The GenWay ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

12.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION
Prod. No.: 40-521-475128 TBE/FSME IgG-ELISA (96 Determinations)
BIBLIOGRAPHY


Tick-Borne Encephalitis (TBE) and its Immunoprophylaxis, IMMUNO AG, Wien (1997)
### Symbols Key

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td><img src="image" alt="Sample diluent buffer IgG" /></td>
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<tr>
<td><img src="image" alt="Stop solution" /></td>
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<tr>
<td><img src="image" alt="TMB Substrate solution" /></td>
<td>TMB Substrate solution</td>
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<tr>
<td><img src="image" alt="Washing solution 20x concentrated" /></td>
<td>Washing solution 20x concentrated</td>
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<tr>
<td><img src="image" alt="Contains sufficient for “n” tests" /></td>
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# SCHEME OF THE ASSAY
TBE/FSME IgG-ELISA

## Assay Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit
Select the required number of microtiter strips or wells and insert them into the holder.

## Assay Procedure

<table>
<thead>
<tr>
<th>Blank (z.B. A1)</th>
<th>Standard</th>
<th>Sample (1+100 prediluted)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Standard A</td>
<td>-</td>
<td>100µl</td>
</tr>
<tr>
<td>Standard B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard C</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Standard D</td>
<td>-</td>
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<tr>
<td>Standard E</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample (1+100 prediluted)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit
**Incubate for 1 h at 37°C**
Wash each well three times with 300µl of washing solution

Conjugate | - | 100µl | 100µl | 100µl | 100µl | 100µl |

Cover wells with foil supplied in the kit
**Incubate for 30 min at room temperature**
Wash each well three times with 300µl of washing solution

<table>
<thead>
<tr>
<th>TMB</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
</tr>
</thead>
</table>

**Incubate for 15 min at room temperature in the dark**

Stop Solution | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl |

Photometric measurement at 450 nm (reference wavelength: 620 nm)

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