Recombinant Tick-borne encephalitis virus Envelope Protein E

Introduction

For many years, enzyme-linked immunosorbent assay (ELISA) was routinely used for in-vitro diagnostics of infectious diseases. As advanced automation entered routine laboratories, and new needs for flexibility in testing arose, chemiluminescent immunoassays (CLIA) became more and more popular. CLIA technology is a very versatile and reliable method allowing fast and economic testing of patient samples. However, with the increasing complexity of CLIA assays, developers also face new technical challenges, e.g. demands for higher quality of raw materials or optimization of bead coupling.

Virion\Serion's recombinant **Tick-borne encephalitis virus (TBEV) Envelope E** antigen **(BA112R01)** is produced for optimized CLIA performance.

The protein production in an ISO 13485 certified environment guarantees the expected high quality, traceability and lot-to-lot consistency paired with readily available bulk quantities.

Background

TBEV belongs to the family *Flaviviridae* of positive-sense, single-stranded RNA viruses and is considered to be the medically most important arthropod-borne virus in Europe. Tick-borne encephalitis (TBE) is a human viral infectious disease involving the central nervous system, and occurring in many parts of Europe and Asia. The virus is transmitted by the bite of infected ticks, found in woodland habitats. TBE is most often manifested as a two-phased illness. The first phase is associated with symptoms like fever, fatigue, headache, muscular ache and nausea. The second phase involves the

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neurological system with symptoms of meningitis (inflammation of the membrane that surrounds the brain and spinal cord) and/or encephalitis (inflammation of the brain), which can result in long-term neurological symptoms, and even death. The exact determinants of disease severity are not known. Like other tick-borne infectious diseases, the risk from TBE can be reduced by using insect repellents and protective clothing to prevent tick bites. A vaccine is available in some disease endemic areas.

TBEV serological tests are commonly used for confirmation of acute infection, control of vaccination effectiveness and support of epidemiological studies.

TBEV Envelope protein E is highly expressed on TBEV viral particles as well as on infected cells. It is highly immunogenic and plays a major role in the biology of the infection. It binds to the host cell surface receptor, mediates fusion between viral and cellular membranes and constitutes the main target for neutralizing antibodies. (1, 2)

Order Information and Related Products

Code	Description	Packaging
BA112R01 *NEW!*	**Tick-borne encephalitis virus Envelope protein E** Envelope protein E of Tick-borne encephalitis virus (TBEV) Source: recombinant protein, expressed in insect cells Molecular weight: 46 kDa, Affinity tag: Strep-tag	1 mg
PLS112G	Tick-borne encephalitis Virus IgG positive defibrinated human plasma	1 mL
PLS112M	Tick-borne encephalitis Virus IgM positive defibrinated human plasma	1 mL
PLS112GN	Tick-borne encephalitis Virus IgG negative defibrinated human plasma	1 mL

Material and Methods

Antigen

Virion\Serion's TBEV Envelope protein E (Far Eastern subtype, strain Sofjin) is recombinantly produced in insect cells and highly purified by strep-tag affinity chromatography, which guarantees high quality and availability. Read on to learn more about efficient IgG and IgM detection with the new TBEV Envelope protein E (BA112R01).

Immunoassay

Recombinant TBEV Envelope protein E antigen (BA112R01) was immobilized on magnetic beads with 4 µm in diameter, similar to those which are commonly used for CLIA assays. The coupling of the antigens as well as the subsequent immunoassays were performed in an automated workflow on a KingFisher™ Flex System (ThermoFisher Scientific™). For IgG or IgM detection, antigen coupled

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magnetic particles were incubated with diluted human sera for 20 minutes at 37°C and then washed. Bound anti-TBEV IgG or IgM antibodies were detected after 20 minutes incubation at 37°C with fluorescently labeled secondary antibody by using a Cytoflex S flow cytometer (Beckman Coulter). Reference ELISA assays were performed according to manufacturer's instructions.

Dynamic light scattering and size exclusion chromatography

Dynamic light scattering (DLS) on a Zetasizer Ultra (Malvern Panalytical GmbH) was used for the measurement of size distribution and the detection of aggregates of the different TBEV Envelope protein E lots in solution.

Size exclusion-high-performance liquid chromatography (HPLC-SEC) is a high-throughput analytical method that allows to determine and quantify the level of aggregates and fragments of purified proteins. HPLC-SEC was performed using a Superdex increase 200 3.2/300 SEC column (Cytiva) on a 1260 Infinity II LC System (Agilent Technologies Sales & Services GmbH & Co.KG).

Glycosylation assay

TBEV Envelope protein samples were treated with 500.000 Units (U)/mL PNGase F according to manufacturer instructions to analyze glycosylation. Denatured protein samples were separated by their molecular weight using SDS-PAGE and afterwards visualized by Coomassie staining.

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Results

Biochemical Analysis

Three independent lots of recombinant TBEV Envelope protein E were analyzed using a size exclusion-high-performance liquid chromatography (HPLC-SEC). As shown in Figure 1, the TBEV Envelope protein E lots display a mean molecular weight of 123 kDa with < 1% aggregates or higher oligomers as determined by peak retention time analysis at 280 nm detection wavelength. This is 2.6 times the calculated molecular weight (ProtParam, www.expasy.org) of the unglycosylated monomer. Considering glycosylation (see glycosylation analysis below), this weight indicates dimeraziation of the protein.

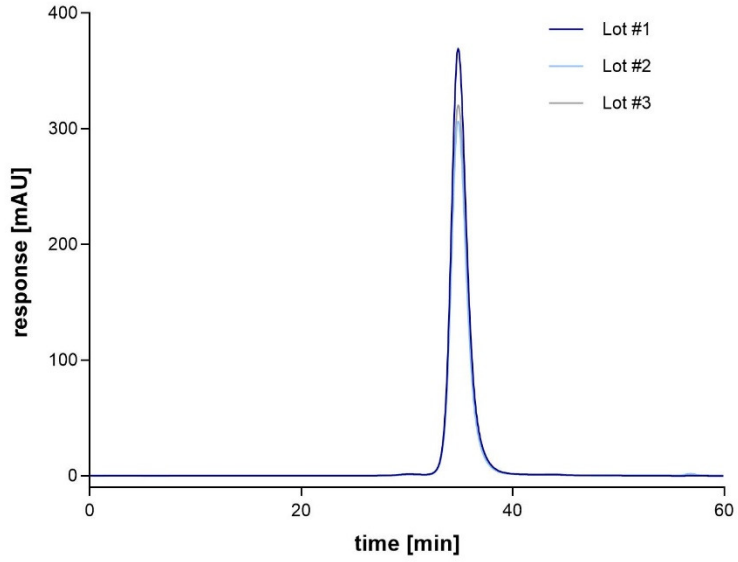

Figure 1: Analytic HPLC-SEC of three independent lots of recombinant TBEV Envelope protein using a Superdex increase 200 3.2/300 SEC (Cytiva) column. Peak retention time (detection at 280 nm) indicates a size of 123 kDa pointing to dimerization and glycosylation of the protein.

To verify homogeneity, three independent lots of recombinant TBEV Envelope protein E were analyzed using dynamic light scattering (DLS). Figure 2 shows the comparison of the three peaks highlighting a strong consistency and homogeneity with < 1% aggregates detected by DLS in all lots. In addition, the hydrodynamic diameter was determined to be 9.2 nm.

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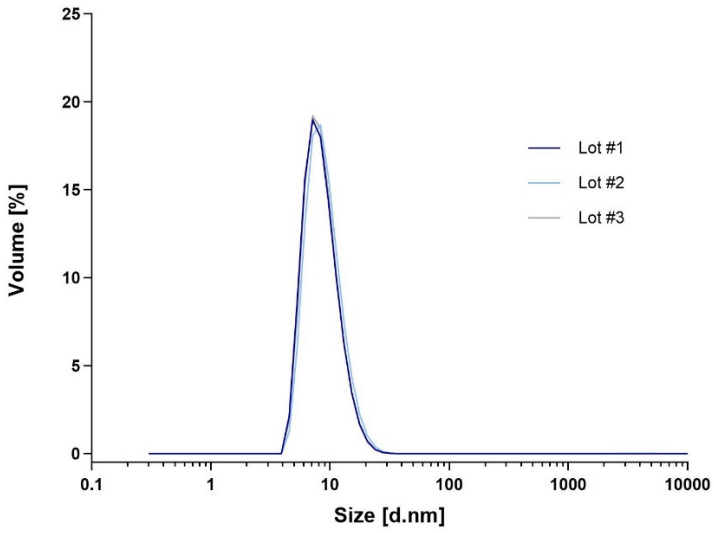

Figure 2: DLS analysis of three independent lots of recombinant TBEV Envelope protein. Peak overlay illustrates a high conformity. The hydrodynamic diameter is 9.2 nm.

In addition three independent lots were compared by SDS-PAGE. The excellent lot to lot consistency is shown in Figure 3. In summary, these analytical methods demonstrate a high level of purity and homogeneity within the different lots.

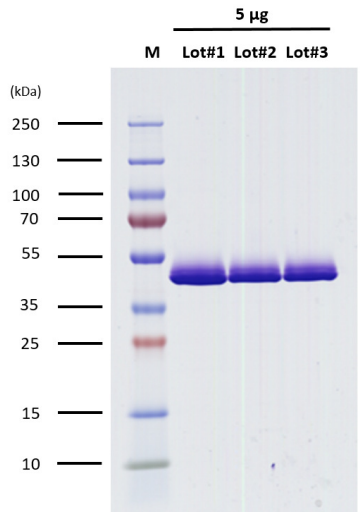

Figure 3: Comparison of three independent lots of TBEV Envelope Protein E by SDS-Page, stained with Coomassie solution.

Glycosylation of recombinant TBEV Envelope protein E was further assessed by PNGase F treatment. The enzyme PNGase cleaves glycoside bonds leading to glycan-free proteins. As shown in Figure 4,

SDS-PAGE analysis revealed a band shift in the PNGase F treated sample confirming glycosylation of the untreated protein.

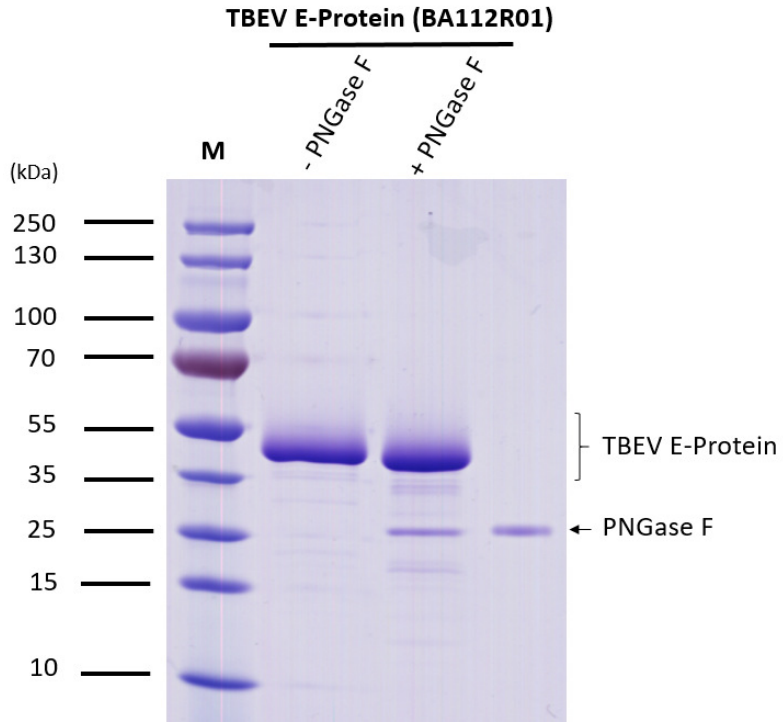

Figure 4: Analysis of glycosylation. TBEV Envelope protein E was incubated with 500.000 U/mL PNGase F according to manufacturer instructions, analyzed by SDS PAGE and stained with Coomassie solution. Band shift in PNGase F treated sample confirms glycosylation of TBEV Envelope protein (exemplarily shown for Lot #1).

Lot to Lot Consistency

Binding of IgG and IgM antibodies by two independent lots of TBEV Envelope protein E was assessed by immunoassay with a fluorescence readout using a flow cytometer. Therefore 47 human samples for IgG and 45 human samples for IgM, covering a wide range from TBEV IgG or IgM negative to highly positive, were evaluated.

As shown in Figure 5, very high correlation coefficients of $r > 0.99$ could be achieved, underlining a high lot to lot correlation. The observed slopes for IgG (1.02) and IgM (1.04) indicate same reactivity for both antigen lots. In summary, the high reproducibility proves excellent lot to lot consistency.

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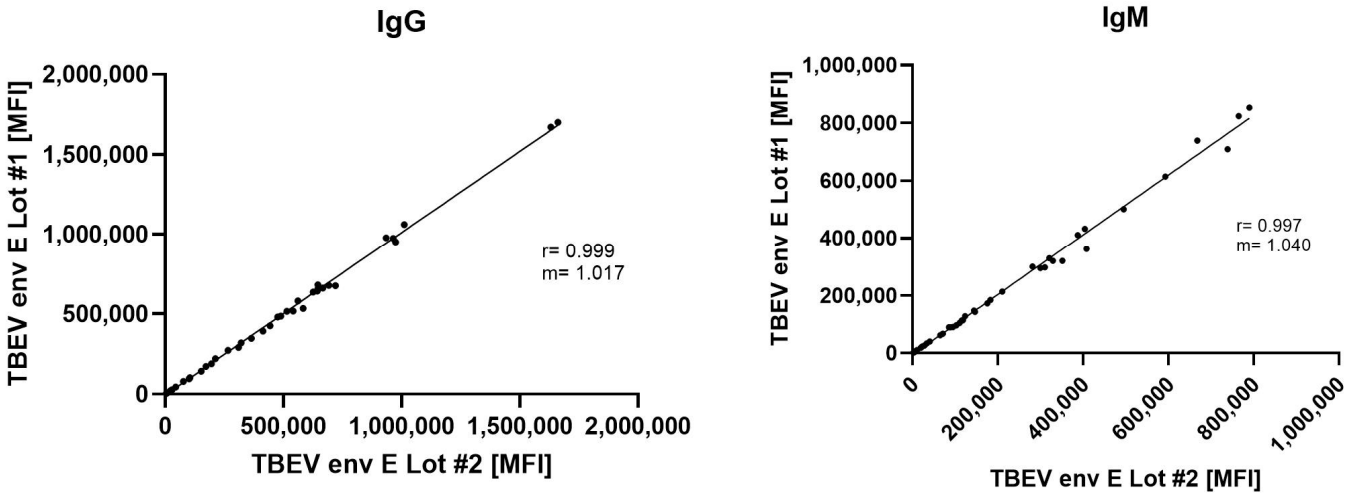

Figure 5: Comparison of independent lots of TBEV Envelope Protein E by conducting an IgG and IgM immunoassay. Statistical analysis was performed using the Passing-Bablok regression. Pearson correlation coefficient r = 0.999 (IgG) and r = 0.997 (IgM) as well as slope m= 1.017 (IgG) and m= 1.040 (IgM).

Reactivity and Measurement Range

In order to evaluate the reactivity of IgG and IgM antibodies towards the recombinant TBEV envelope protein E and to estimate the measurement range, a comparison with the native TBEV antigen was conducted using a bead-based immunoassay.

Consequently, 84 human serum samples, comprising 45 positive and 39 negative samples, were subjected to IgG analysis. The observed median reactivity for recombinant TBEV envelope protein E was 472,000 MFI, in comparison to 192,000 MFI for native TBEV antigen. This indicates a wide measurement range (Figure 6). The ratio between the mean value of the positive samples and the mean value of the negative samples (p/n ratio) was 10.2 for the recombinant TBEV envelope protein E and 17.6 for the native antigen. Both antigens exhibited high IgG reactivity. However, the relatively high mean value of the negative samples (88,000 MFI for the recombinant antigen) suggests that the serum samples should be diluted further to achieve a higher p/n ratio.

A total of 80 human samples were subjected to IgM analysis, of which 33 exhibited positive results and 47 displayed negative outcomes. The median reactivity observed for the recombinant TBEV envelope protein E was 15,600 MFI, in comparison to 11,500 MFI for the native TBEV antigen (Figure 6). The p/n ratio was 20.0 for the recombinant TBEV envelope protein E and 5.67 for the native antigen. In conclusion, the recombinant TBEV envelope protein E demonstrates excellent IgM reactivity and a broad measuring range.

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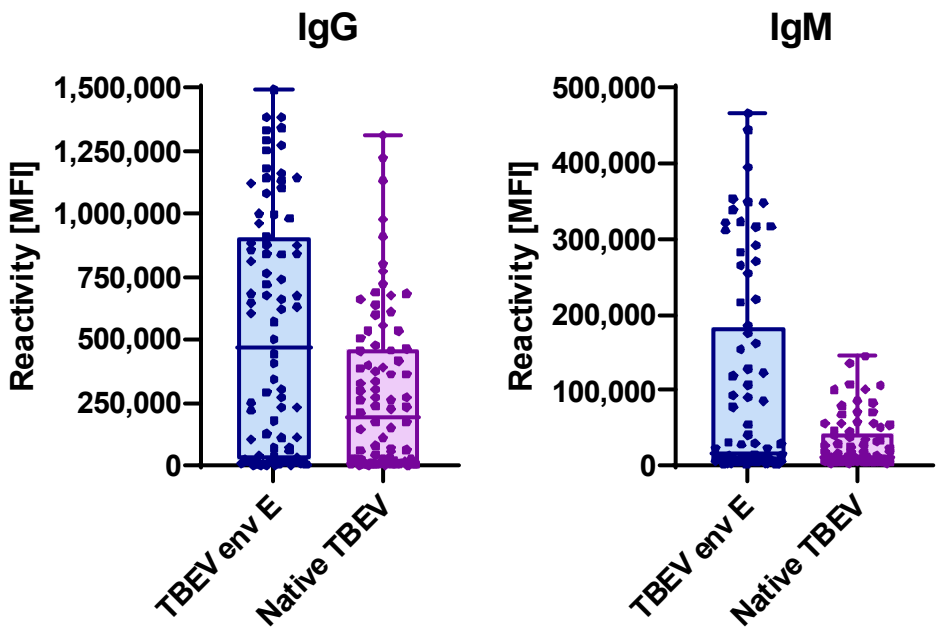

Figure 6: A comparative analysis of recombinant and native TBEV antigens. An IgG immunoassay was conducted on 84 human samples, comprising 45 positive and 39 negative samples. The observed median reactivity of all samples was 472,000 median fluorescence intensity (MFI) for the recombinant TBEV envelope protein E and 192,000 MFI for the native antigen. An IgM immunoassay was conducted on 80 human samples, comprising 33 positive and 47 negative samples. The observed median reactivity was 15,600 MFI for the recombinant TBEV envelope protein E and 11,500 MFI for the native antigen.

Diagnostic Performance: Sensitivity and Specificity

A comparative analysis was undertaken to assess the diagnostic efficacy of recombinant and native TBEV antigens for the detection of IgG and IgM antibodies. An IgG bead-based immunoassay was conducted on 84 human samples, of which 45 were positive and 39 negative. The positive and negative status of the samples was confirmed by two commercial TBEV IgG assays based on a native antigen. A receiver operating characteristic (ROC) analysis revealed an area under the curve (AUC) of 0.990 for the recombinant TBEV envelope protein E and 0.996 for the native antigen (Figure 7), indicating excellent diagnostic performance for both antigens.

An IgM bead-based immunoassay was conducted on 80 human samples, comprising 33 positive and 47 negative samples, which were tested with three commercial IgM assays based on a native antigen. The AUC was 0.995 for the recombinant TBEV and 0.964 for the native antigen (Figure 7). Once more, the recombinant TBEV envelope protein E was demonstrated to be perfectly suited for IgM detection.

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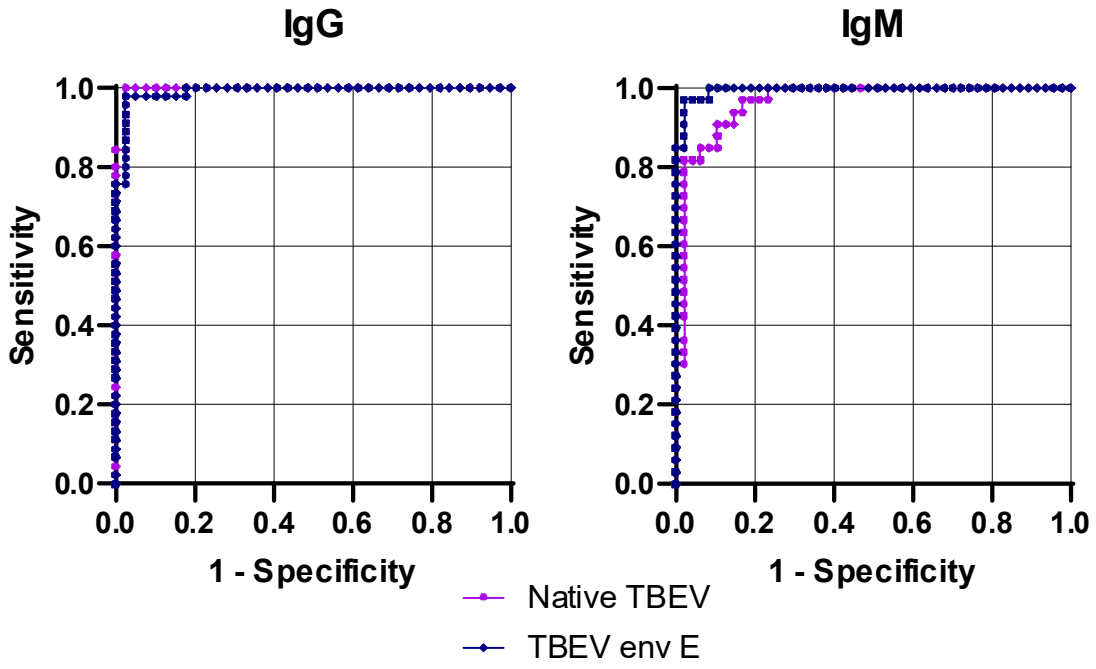

Figure 7: A diagnostic comparison was conducted between recombinant and native TBEV antigen. An IgG immunoassay was performed with 84 human samples, 45 of which were positive and 39 negative. The receiver operating characteristic (ROC) analysis revealed an area under the curve (AUC) of 0.990 for the recombinant TBEV and 0.996 for the native antigen. An IgM immunoassay was performed on 80 human samples, comprising 33 positive and 47 negative samples. The AUC was 0.995 for the recombinant TBEV and 0.964 for the native antigen.

In order to determine the sensitivity and specificity of recombinant TBEV envelope protein E, an ideal threshold was defined in such a way as to minimise the number of false positive and false negative results. For IgG, this optimised cut-off value was 302,000 MFI, again indicating that serum samples should be diluted further. Nevertheless, the IgG immunoassays demonstrated a high level of sensitivity (97.8%) and specificity (97.4%) (Table 1).

The results of the IgM immunoassay showed an optimal threshold at 31,000 MFI, indicating that the sensitivity (97.0%) and specificity (97.9%) were very similar to those observed for IgG (Table 1).

Table 1: Recombinant TBEV Envelope protein E: sensitivity and specificity were determined for IgG (n = 84) and IgM (n = 80).

	Sensitivity	Specificity
IgG	97.8 %	97.4 %
IgM	97.0 %	97.9 %

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Cross Reactivity

Viruses of the *Flaviviridae* family are known for their cross-reactivity in serological tests. Here, samples positive for IgG or IgM to dengue virus, West Nile virus or Zika virus were analysed using the recombinant TBEV envelope protein E bead-based immunoassay and commercial TBEV ELISA. The overall agreement and disagreement are shown in Table 2.

Out of a total of 38 samples tested for IgG, 27 were either positive or negative on both tests. However, 11 samples remained positive by ELISA, whereas these samples were negative by recombinant TBEV envelope protein E immunoassay. It is noteworthy that 10 of these discrepant samples were West Nile virus positive samples.

For IgM, 39 samples were tested and all were negative by TBEV ELISA. In the recombinant TBEV envelope protein E immunoassay, 5 samples were positive with signals up to 1.5 times above the cut-off.

The number of samples used here was quite small, but the results seem to reveal potential different characteristics of the recombinant TBEV envelope protein E and the native TBEV antigen.

Table 2: Potentially cross-reactive samples from other Flaviviridae and blood donors were tested for the presence of IgM and IgG using the recombinant TBEV envelope protein E bead-based immunoassay and commercial TBEV ELISA.

		IgG ELISA		IgM ELISA	
		pos/eq	neg	pos/eq	neg
TBEV env E	pos	16	0	0	5
	neg	11	11	0	34

Summary

Our recombinant TBEV Envelope Protein E (BA112R01) is a highly purified protein, which guarantees a high quality and availability. Moreover, a great lot to lot consistency is achieved. It is well suited as reactive and reliable candidate for efficient IgG and IgM detection in serological IVD assays. Especially the high performance on beads makes TBEV Envelope protein E antigen a promising candidate for diagnostic serology tools.

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