Technical Data Sheet

InVivoMAb anti-Eastern equine encephalitis virus E2 protein



<u>Attention</u>: Use of this product constitutes an agreement to Bio X Cell's Terms and Conditions which are included with this product in print and can also be found at https://bioxcell.com/terms-and-conditions.

Lot Specific Information

Lot Number: Lot Specific* Volume: Lot Specific*

Concentration: Lot Specific* (generally 4 to 11 mg/ml) *

Total Protein: Lot Specific*

*This information will be noted on the certificate of analysis that ships with this product.

Product Information

Catalog Number: BE0433 Clone: EEEV-3

Isotype: Mouse IgG2c, κ

Recommended Isotype Control(s): InVivoMAb mouse IgG2c isotype control, anti-dengue virus

Recommended Dilution Buffer: InVivoPure pH 7.0 Dilution Buffer

Immunogen: SINV-EEEV

Reported Applications: in vivo protection against EEEV

in vitro neutralization of EEEV

Flow cytometry

ELISA

Plasma membrane fusion-from-without (FFWO) Focus reduction neutralization tests (FRNT)

Biolayer Interferometry (BLI)

Formulation: PBS, pH 7.0

Contains no stabilizers or preservatives

Endotoxin: <2EU/mg (<0.002EU/μg)

Determined by LAL gel clotting assay

Purity: >95%

Determined by SDS-PAGE

Sterility: 0.2 µm filtration

Production: Purified from cell culture supernatant in an animal-free facility

Purification: Protein A

Molecular Weight: 150 kDa

Description

The EEEV-3 monoclonal antibody reacts with the B domain of the E2 glycoprotein on the eastern equine encephalitis virus (EEEV). In humans and horses, EEEV is transmitted primarily through the bite of the swamp mosquito Culiseta melanura (i.e., black-tailed mosquito). Infected mosquitoes transmit the EEEV to birds as well, and uninfected mosquitoes acquire the EEEV infection through feeding on infected birds. Human EEEV infection incidences are low, but the mortality rate can be up to 70%, and the survivors end up with significant brain damage. Structurally, the EEEV is an enveloped virus with single-stranded positive sense RNA, and the mature virion displays spikes that are made of E2-E1 heterodimers. The E2 glycoprotein binds to some host cell receptors, and it is suggested to be responsible for viral entry and endocytosis. In experimental studies, antibodies against epitopes on the E2 protein (e.g., EEEV-3) are used to elucidate the molecular mechanism of EEEV infections. The EEEV-3 monoclonal antibody is specific for EEEV's E2 glycoprotein (B domain), and it does not bind other related viruses such as the Western equine encephalitis virus (WEEV) and the Venezuelan equine encephalitis virus (VEEV). Cryo-EM structure analysis and alanine-scanning mutagenesis studies demonstrated that the Fab fragments of the EEEV-3 monoclonal antibody interact exclusively with an epitope localized to residues 180–182 on the B

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domain of the EEEV E2 glycoprotein. In vitro experiments have shown that the EEEV-3 monoclonal antibody exhibits a modest inhibition of viral attachment to the plasma membrane of the cells. Post-attachment neutralization assays showed the in vitro inhibitory activity of the EEEV-3 monoclonal antibody against SINV-EEEV infection when the antibody was added after the virus was bound to cells. In the FFWO assay, the EEEV-3 monoclonal antibody was added after viral attachment to Vero cells, and it was found that the antibody significantly blocks the virus plasma membrane fusion. FRNT assays involving the infection of Vero cells with EEEV particles that were pre-incubated in vitro with the EEEV-3 monoclonal antibody or its Fab fragments demonstrated that the bivalent mouse IgG monoclonal antibodies efficiently inhibit infection while the Fab fragments does not. The in vivo efficacy of the EEEV-3 monoclonal antibody was evaluated in CD-1 mice, which were inoculated with the highly pathogenic EEEV strain FL93-939. A single 100-µg i.p. injection of the EEEV-3 monoclonal antibody one day before or after subcutaneous or aerosol inoculation of EEEV (lethal doses) provided significant protection against viral infection.

Storage

Store at the stock concentration at 4°C. **Do not freeze.**

It is not uncommon for a floccule or precipitate to appear during storage. The floccule is typically buffer salts precipitating out of solution or a small bit of protein aggregation. For information on how to remove floccules or precipitates see our FAQ's at https://bioxcell.com/fags.

Protocol Information

Since applications vary, each investigator should use the application references as a guide to help estimate the appropriate dose or concentration. The dose or concentration can be further optimized experimentally in a dose response or titration experiment.

Application References

For a complete list of references, visit https://bioxcell.com/catalogsearch/result/?q=BE0433#tab_references or scan the QR code below.



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