EVEC, Inc

Antibody Datasheet

Product name
Human Metapneumovirus F glycoprotein antibody (5-12C)

Product description
Complement-independent neutralizing monoclonal antibody to hMPV F protein

Catalog Number
EVHM0501-100

Source
Human (recombinant production in CHO-K1)

Clonality and Clone Name
Monoclonal, 5-12C

Isotype
IgG1 Lambda

Form Supplied and Size
Liquid, 100 μg

Concentration and Storage Buffer
1 mg/mL in Phosphate buffer saline pH7.4 (containing no preservative)

Storage
Antibody can be kept at 4°C for up to 1 month and should be kept at -20°C or below for long-term storage. To avoid repeated freeze thaw cycles, antibody should be aliquoted before frozen.

Purification
Purified by protein A chromatography. The purity is greater than 95% by SDS-PAGE.

Antigen for Screening
Recombinant hMPV F protein-expressing CHO-K1.

Specificity
Clone 5-12C does not cross-react with cell cultures infected with Human Respiratory Syncytial Virus (hRSV).

Epitope
Epitope has not been determined.

Applications
ICC/IF, FCM, Neutralization assay. Other applications have not been tested.

Limitations
This product is to be used for research purposes only.

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Background information

Human metapneumovirus (hMPV) is a member of the pneumovirinae subfamily of paramyxoviruses, first discovered in 2001. hMPV is a common cause of respiratory infection. hMPV is associated with the common cold in the upper respiratory tract infection, while lower respiratory tract infection can cause severe illnesses such as pneumonia and bronchiolitis in the very young, the very old or immunosuppressed patients. There are two major serotypes, A and B, each containing two sub-groups (A1, A2, B1 and B2).

hMPV F glycoprotein expressed on the cell surface can mediate fusion with neighboring cells to form syncytia.

Immunogen and Recombinant Production Host

This antibody was generated from a healthy individual by a method based on the Epstein-Barr virus transformation of peripheral blood mononuclear cells followed by the isolation of antibody-producing cells. The antibody reactivity for the target antigen was screened by immunofluorescent staining by using CHO-K1 cells displaying recombinant hMPV F protein. The antibody genes were cloned from the antibody-producing cells and introduced into CHO-K1 cells for antibody production.

Application Note

Recommended Starting Dilutions:

For ICC/IF: Use at 1:500 – 1:4000

Not yet tested in other applications

The optimal working dilution should be determined experimentally by the end user.

Neutralization assay

The 50% of inhibitory concentration (IC_{50}) values of the antibody against hMPV-A and -B were ≥ 100 ng/mL and ≥ 300 ng/mL, respectively.

The IC_{50} was determined as described below.

1. Plate LL-MK2 cells in a tissue plate with growth medium, and incubate at 37 °C, 5% CO_2.
2. Check the plate to confirm 100% confluency and even cell distribution.
3. Remove culture medium from plate and wash each well twice with assay medium.
4. Prepare serial dilution of antibodies and mix with an equal volume of virus diluent. Include the following controls: no antibody control, normal IgG control and no virus control in defined wells.
5. Gently agitate the virus-antibody mixtures, and incubate for 1 hour at 25 °C, 5% CO_2.
6. Transfer co-incubated antibody-virus mixture to wells containing monolayers of the target cells.
7. Incubate for 1 hour at 37 °C, 5% CO_2.
8. Remove culture medium from each well and wash each well three times with assay medium.
9. Add assay medium to each well.
10. Incubate the plate under appropriate condition.
11. Remove assay medium from the well.
12. Fix cells and detect virus-infected cells by immunofluorescent staining for the determination of IC_{50}.

Consult the available literature for the best system for your intended assay.

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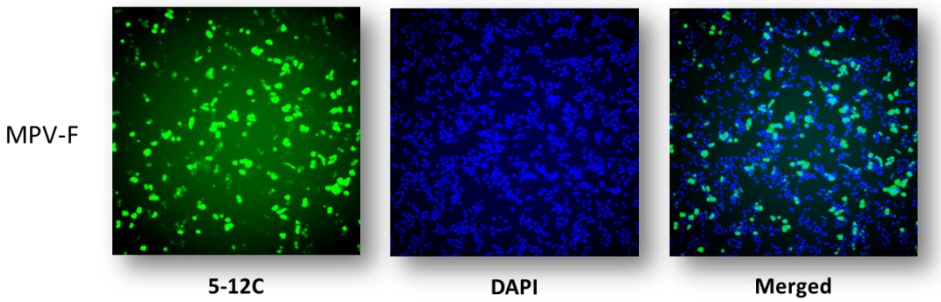
Immunofluorescence Results



Immunofluorescent staining was performed by using MPV-F protein expressing CHO-K1. Brief protocol is described below.

1) Plasmid vector which accommodate MPV-F gene was transfected into CHO-K1.
2) Twenty four hours post-transfection, cells were fixed with 4% paraformaldehyde-PB
3) Cells were permeabilized with 0.2% Tween-PBS for few minutes.
4) 5-12C antibody was diluted and treated as a primary antibody for 1hr.
5) Anti-human IgG (gamma chain)-FITC was treated as a secondary antibody for 1hr.
6) DAPI was diluted and treated for 5 min.
7) Wash with PBS(-) four times.
8) Observe images by fluorescent microscopy.

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