Mouse Hepatitis Virus
MHV IFA

Immunofluorescence Test Kit
For The Detection of MHV Antibodies

Version 1.0
Catalog Number IFA-K101

The XpressBio Mouse Hepatitis Virus (MHV) Indirect Fluorescent Antibody (IFA) kit is intended to be used as a research tool for the detection and titration of hepatitis virus antibodies in mouse serum.

INTRODUCTION

Mouse Hepatitis Virus (MHV) is a virus of the family *Coronaviridae* and genus *Betacoronavirus.* The *Murine Coronavirus,* Mouse Hepatitis Virus is a coronavirus that causes an epidemic murine illness with high mortality, especially among colonies of laboratory mice. Some strains of MHV cause a progressive demyelinating encephalitis in mice which has been used as a model for multiple sclerosis. MHV may be transmitted through aerosols, fomites, and direct contact. The virus is highly contagious, although not persistent in the environment. The effects of MHV on research are many, however MHV can infect the lymphatic tissue and therefore has significant and prolonged effects on the immune system, even in immunocompetent mice. Coinfection with MHV can modulate the course of several viral, bacterial, and parasitic infections, resulting in a more severe infection, resistance to infection, or a more or less severe course of infection, depending on the particular agent. MHV may also modify the activity of hepatic enzymes, reduce liver regeneration after partial hepatectomy, produce anemia, leukopenia, and thrombocytopenia, and decrease the incidence of diabetes in some susceptible strains of mice. MHV is extremely contagious and is one of the most frequent viral infections found in modern research facilities.

KIT CONTENTS

Product	Catalogue	Per Kit
MHV IFA Slide, 8 well 7 mm	IFA-S101	12
Blocking Buffer	IFA-Block	2 x 3 ml
MHV Positive Control	IFA-PC101	1.5 ml
Mouse Normal Control	IFA-NC100	1.5 ml
Fluorescein Conjugate (500-1000x)	IFA-FC	10 µl
Phosphate Buffer Saline Powder	IFA-PBS	3 Packages
Sample/Conjugate Diluent Buffer	IFA-SCD	10 ml
Mounting Solution	IFA-Mount	3 ml
Slide Cover Slips	IFA-CS	12
Slide Blotters	IFA-Blotters	12
Instruction Manual	NA	1

TECHNICAL ASSISTANCE

Please refer any technical questions to info@xpressbio.com.

SAFETY INFORMATION

Sodium azide may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water. The MSDS for this kit is available online at www.expressbiotech.com.

STORAGE CONDITIONS

The MHV IFA Kit can be stored at 2-8ºC until the expiration date on the kit. **Remove the tube of conjugate from the kit and store at -20º C.** The packets of PBS, cover glass, absorbent blotters, mounting solution, and product insert can be stored at room temperature.

REAGENTS AND EQUIPMENT SUPPLIED BY THE USER

- Pipettes, tubes, and sterile tips for dilutions
- Disposable laboratory gloves
- Humidified chamber (optional)
- Sterile distilled (deionized) water
- Magnetic stir plate (optional)
- Staining dish and slide-holder rack
- Fluorescent microscope equipped with Rhodamine or FITC filters

NOTES BEFORE STARTING AND KIT CONTENTS

Carefully review the protocol before beginning the test. Each well on a slide contains a cell monolayer that has been infected with MHV. The percentage of viral infected cells in a well varies from 20-40% and the uninfected cells serve to contrast the intense nuclear staining of MHV infected cells. If performing the incubation steps at 37º C instead of 25º C the incubation time can be reduced to 15-20 minutes. Each lot of XpressBio's MHV IFA Kit has been extensively tested and the conditions under which the kit is shipped and stored have been shown empirically to not impact assay performance.

PBS Wash Buffer and Slide Washing. PBS Wash Buffer is provided as a dry powder. Add the contents of one package to a one liter (1 L) storage bottle filled with distilled or deionized water. Warm to dissolve the powder faster. Store the PBS at room temperature for up to a month. Removing reagents from the wells of a slide between steps can be accomplished in the following ways: 1) a gentle flow stream of PBS from a wash bottle, not hitting the wells directly; 2) dumping or tapping a slide on its edge into a layer of paper towels (Block Buffer and conjugate may be the same in all wells) and immediately immerse into a PBS bath; 3) remove the liquid from each well using a pipet and a fresh tip per well; 4) aspirate with a fine tip connected to a pump.

Blocking Buffer. Blocking Buffer is provided as a 1x ready to use liquid in a 3 ml dropper bottle that delivers a 30-50 µl drop per application. Store Blocking Buffer at 4º C after use.

Sample and Conjugate Diluent Buffer. Sample/conjugate diluent buffer is supplied as a 1x ready to use solution for the dilution of mouse test serum samples and conjugates. One point dilutions of serum samples can be made at 1:15, and serial dilutions can be made to titrate the test serum in order to estimate antibody titer. Conjugate is supplied tagged with FITC or Rhodamine and are diluted 1:1000 with diluent buffer. Store the diluent buffer at 4º C after use.

Positive Control and Normal Control.

The Positive Control and Normal Control are supplied as a 1x ready to use liquid in 3 ml dropper bottles that deliver 30-50 µl per application. Store at 4° C after use.

MHV IFA KIT PROTOCOL

1. Warm Kit Reagents to Room Temperature.

Remove the number slides required and the reagents from the kit and allow them to warm to room temperature. Return the extra slides and kit to 4° C. All subsequent steps are performed at room temperature.

2. Hydration of Wells and Optional Blocking.

Place slides in rack and immerse in PBS for 2 min. Blot dry the area around each well with 8-well blotting paper (cotton tipped swabs work) without letting the wells dry out. Place the slides in a small pan/tray with absorbent paper on the bottom. Add a 30-50 µl drop of Blocking Buffer to every well and incubate for 15 min. Remove the blocking reagent from wells as described above under the PBS wash buffer section. Incubate in a PBS bath for 2 minutes and then blot/dry the mask area around the wells.

3. Application of Positive, Negative, and Test Samples.

The positive control (Mouse Anti-MHV serum) and negative control (normal mouse serum) are supplied ready to use in a 3 ml dropper bottle. Add one drop of each solution to two different wells in order to control the slide. Test serum samples are diluted in serum diluent at a 1:25 dilution or can be titrated to look for an end point dilution of the sample. Incubate the slide with samples in the wells at room temperature for 30 minutes. Wash the samples from the wells as described in the PBS wash buffer section and incubate in a 50-400 ml bath of PBS for 10 minutes. Repeat the wash step a second time. Blot/dry the mask of the slide with blotter paper, trying not to let the well dry out.

4. Staining Cells with Rhodamine- or FITC-Conjugate.

Conjugate is supplied as a concentrated solution that should be stored at -20° C and diluted to 1:1000 in sample/conjugate dilution buffer just before use. Add one drop or 30-50 µl of conjugate to each well and incubate at room temperature for 30 minutes. Dump the conjugate from the wells onto a paper towel and wash in three (3) changes of PBS buffer incubating each wash step for 5-10 minutes. Blot the mask of the slide dry with blotter paper, add a few drops of mounting solution, and apply a cover slip over all eight wells.

5. Score Wells with a Fluorescent Microscope.

For best results, the slide should be read immediately at a magnification of 200-500x. Alternatively, the slides may be read within 24 hours, however, they should be stored at 2-8° C in the dark, and the cover glass should be sealed to prevent the mounting medium from drying out. The negative control well demonstrates a week homogeneous staining pattern throughout the cell. The MHV positive control demonstrates an intense (20-100x the negative control staining) homogeneous staining pattern with strong nuclear staining. In addition, cells contain MHV at different stages of the virus life cycle and areas of intense virus staining can be seen at the cytoplasmic membrane of large cells.

EXPERIENCED USERS PROTOCOL

1. Warm slides and reagents to room temperature. All Subsequent steps are performed at room temperature.
2. Hydrate slide wells in PBS bath for 2 minutes and blot the slide mask dry (use slide blotters or cotton tip swabs to dry the area around each well).
3. Apply 30-50 µL of IFA Block buffer to each well and incubate for 15 minutes (optional step).
4. Dump the majority of the block buffer from the slide using a paper towel, place each slide in the rack of a staining dish filled with PBS and incubate for 2 minutes. Blot/dry the slide mask around each of the wells.
5. Apply a 30-50 µl drop of Negative and Positive Control to wells 1and 2 and to wells 3-8 add 30-50 µl of unknown test samples. Incubate for 30 minutes.
6. Remove the contents of each well with: 1) a pipet and fresh tip per well; or 2) a gentle stream of PBS from a wash bottle; or 3) aspirate with a fine tip connected to a pump; or 4) dump onto several layers of paper towel and immediately immerse into a PBS bath.
7. Place slide in rack/dish and wash in PBS for 5-10 minutes while stirring gently on a magnetic stir plate or equivalent. Repeat PBS wash a second time for 5-10 minutes and blot the mask dry around wells.
8. Apply a 30-50 µl drop of FITC conjugate and incubate at room temperature for 30 minutes.
9. Wash in PBS bath with agitation three (3) times for 5-10 minutes each incubation. Blot mask dry.
10. Before the wells dry out add a few drops of mounting solution and press a glass cover slip onto the slide covering the wells. Examine the wells with a fluorescent microscope within 24 hours or seal the coverslip to the Slide with clear finger nail polish for long term storage. Read the slides with a fluorescent microscope at 200-500x magnification.

CONTACT INFORMATION

Express Biotech International
503 Gateway Drive West
Thurmont, MD 21788 USA
Toll free: 888-562-8914

www.XpressBio.com
info@XpressBio.com
Tel: 301-228-2444
Fax: 301-560-6570