

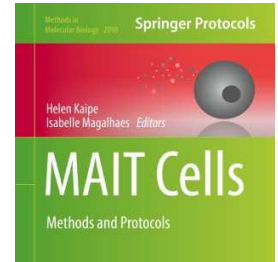
Sep 22, 2021

Influenza A Virus Infection

 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.bmgyk3xw>



Timothy S. C. Hinks¹, Bonnie van Wilgenburg², Huimeng Wang³, Liyen Loh³, Marios Koutsakos³, Katherine Kedzierska³, Alexandra J. Corbett³, Zhenjun Chen³

¹Respiratory Medicine Unit, Nuffield Department of Medicine Experimental Medicine, University of Oxford, Oxfordshire, UK;

²Peter Medawar Building for Pathogen Research and Translational Gastroenterology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK;

³Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Australia

Springer Nature Books



Satyavati Kharde

Springer Nature

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bmgyk3xw>

External link: https://link.springer.com/protocol/10.1007/978-1-0716-0207-2_17

Protocol Citation: Timothy S. C. Hinks, Bonnie van Wilgenburg, Huimeng Wang, Liyen Loh, Marios Koutsakos, Katherine Kedzierska, Alexandra J. Corbett, Zhenjun Chen 2021. Influenza A Virus Infection. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bmgyk3xw>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: September 17, 2020

Last Modified: September 22, 2021

Protocol Integer ID: 42232

Keywords: Virus, MAIT cell, Flow cytometry, MR1-tetramer, Infection, Mouse , pulmonary mait cell, synthetic mait cell antigen, combination of synthetic mait cell antigen, study of mait cell activation, mait cell activation, viral infections in vivo, mait cell, influenza, viral infection, intracellular cytokine, cytokine, range of viral infection, specific pathogen, virus infection, independent manner via cytokine, like lymphocyte, murine model, methods for lung homogenization, lung homogenization, infection,

Abstract

This is part 3.3 of the "Study of MAIT Cell Activation in Viral Infections In Vivo" collection of protocols.

Collection Abstract: MAIT cells are abundant, highly evolutionarily conserved innate-like lymphocytes expressing a semi-invariant T cell receptor (TCR), which recognizes microbially derived small intermediate molecules from the riboflavin biosynthetic pathway. However, in addition to their TCR-mediated functions they can also be activated in a TCR-independent manner via cytokines including IL-12, -15, -18, and type I interferon. Emerging data suggest that they are expanded and activated by a range of viral infections, and significantly that they can contribute to a protective anti-viral response. Here we describe methods used to investigate these anti-viral functions in vivo in murine models. To overcome the technical challenge that MAIT cells are rare in specific pathogen-free laboratory mice, we describe how pulmonary MAIT cells can be expanded using intranasal bacterial infection or a combination of synthetic MAIT cell antigen and TLR agonists. We also describe protocols for adoptive transfer of MAIT cells, methods for lung homogenization for plaque assays, and surface and intracellular cytokine staining to determine MAIT cell activation.



Attachments



Study of MAIT Cell A...

386KB

Materials

For materials, please refer to the Guidelines section of the "[Study of MAIT Cell Activation in Viral Infections In Vivo](#)" collection.

Safety warnings

- ! Personal protective equipment (PPE) should be worn at all times (gloves, lab coat, & eye protection) (see **Notes 3** and **4**).

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).



- 1 Thaw virus vial rapidly in a 37 °C water bath until all ice crystals have melted.
- 2 Decontaminate the outer surface of the vial with 70 % ethanol .
- 3 Perform serial dilutions in sterile PBS to achieve the desired inoculum. For example:
(a) If titer of PR8 stock = 1.0×10^9 plaque-forming units (PFU)/mL, require 25 PFU/inoculum (see **Note 14**).
(b) Volume of inoculum required for intranasal infection = 50 μ L/inoculum.
(c) 25 PFU/50 μ L = 500 PFU/mL, dilution required from virus stock DF = 2,000,000.
 - Dilution 1: 1/100: 10 μ L virus stock + 990 μ L PBS .
 - Dilution 2: 1/100: 10 μ L dilution 1 + 990 μ L PBS .
 - Dilution 3: 1/100: 10 μ L dilution 2 + 990 μ L PBS .
 - Dilution 4: 1/2: (depends on volume required, e.g., 20 inoculations = 1000 μ L)
 500 μ L dilution 3 and 500 μ L PBS .
- 4 Mix virus with vortex before administering the inoculum.
- 5 Infect mice i.n. with 50 μ L under isoflurane anesthesia (see **Note 7**).
- 6 Allow mice to recover and monitor mice for until recovery (typically 10 days) (see **Note 15**).

