

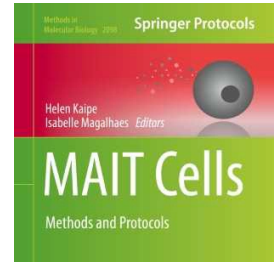
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# MAIT Cell Expansion in Donor Mice

 In 1 collection

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## Abstract

This is part 3.1 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.

**Abstract:** MAIT cells are rare in specific pathogen-free mice [6], typically comprising about  $1 \times 10^4$  recoverable pulmonary MAIT cells in an infection-naïve adult C57BL/6 mouse. Therefore, for adoptive transfer experiments, the MAIT cell population should first be expanded using intranasal infection [15] or immunization (5-OP-RU with TLR agonists) [3, 15] (see Note 5). When planning the adoptive transfer experiment, estimate that one S. Typhimurium BRD509-infected mouse will yield  $1-2 \times 10^6$  sorted MAIT cells, which are enough for 10–20 recipient mice ( $10^5$  MAIT cells/RAG2<sup>-/-</sup>γC<sup>-/-</sup> mouse in this case). Infect donor mice 7 days earlier than the adoptive transfer.

## Attachments



Study of MAIT Cell A...

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## 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 Two days before infection streak out a plate of *S. Typhimurium* BRD509 (an attenuated vaccine strain [14]) on LB agar plates, containing [IM] 50 µg/ml streptomycin and incubate plates Overnight at 37 °C .
- 2 The day before infection, pick a single colony under flame and inoculate to 10 mL LB culture medium with [IM] 50 µg/ml streptomycin and leave static at 37 °C (double contained if working with wild type/virulent SL1344 or equivalent strains) Overnight .
- 3 On the day of infection, re-inoculate into fresh 10 mL pre-warmed LB culture medium with 0.5 µL , 100 µL , or 20 µL of overnight culture, under flame. This is to ensure an optimal optical density (O.D.) reading (bacteria in log phase growth) for preparing the inoculum later (see **Note 6**). The doublingtime for *Salmonella* can vary between 0.5 and 1 h. Make the infection inoculum from culture with O.D.<sub>600nm</sub> reading between 0.2 and 0.6. Calculate the required CFU of bacteria estimating 1 O.D. =  $5\text{--}10 \times 10^8$  CFU (this constant needs to be established for individual labs). Dilute with PBS to a final concentration of  $2 \times 10^7$  CFU/mL, allowing **50 µL inoculum/mouse**, i.e.,  $10^6$  CFU/50 µL/mouse.
- 4 Infect mice i.n. with  $10^6$  CFU *S. Typhimurium* BRD509 in 50 µL PBS under isoflurane anesthesia (see **Notes 7** and **8**).
- 5 Allow mice to recover and monitor mice for 7 days to allow the infection to take its course and MAIT cell frequencies to expand dramatically from  $10^4$  to  $5 \times 10^6$  MAIT cells, or from [IM] 1 % to [IM] 20 % – [IM] 50 % of all alpha-beta T cells [15] (see **Note 8**).