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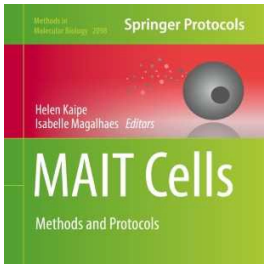
# MAIT Cell Intracellular Cytokine Staining




In 1 collection

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## Abstract

This is part 3.5 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

PDF

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).

## To analyze MAIT cell frequencies and function during viral infection.

1 Prewarm collagenase media and shaking incubator to 37 °C.

2 Mice should be euthanized (e.g., using a rising concentration of $CO_2$ with a second method to confirm death).

3 Open the diaphragm by cutting the rib cage to expose both the heart and lungs. Gently perfuse the right ventricle with 8 mL – 10 mL ice-cold RPMI to remove circulating blood. Perfuse using a 10-mL syringe and a 26-G needle. Proper perfusion will result in lung inflation and a color change to pink/ white.

4 Remove lungs (*see* **Note 18**) using scissors to cut through the hilum and place into a 24-well plate containing ice-cold RPMI to transfer organs to the laboratory.

5 Chop lungs into very small pieces (*see* **Note 9**).

6 Place lung tissue into a 1-mL Eppendorf tube containing 0.5 mL/lung of pre-warmed collagenase/DNase medium. This should also contain, 0.5 µL Brefeldin A (1:1000) (final concentration [M] 3.0 µg/mL).

7 Incubate tubes on their sides in a shaking incubator at 37 °C, at 100 rpm – 180 rpm, for 01:30:00.

8 After 01:30:00 pour digested tissue through a 70-µm cell strainer and force through into Petri dish with the plunger from a 1-mL syringe. Rinse residual cells into a total of 10 mL FACS wash in 10 mL falcon tubes at Room temperature. Centrifuge at 400 x g, Room temperature, 00:05:00.

9 Resuspend in 2 mL per lung (*see* **Note 19**) of pre-warmed TAC lysis buffer at 37 °C. Vortex well, then place in a pre-warmed water bath at 37 °C. After 00:05:00 neutralize by adding an equal volume of FACS buffer. Centrifuge at 400 x g, Room temperature, 00:05:00.

10 Numbers of lung cells can be estimated using a hemocytometer or spectrophotometer (*see* **Notes 20** and **21**).

11 Transfer 100 µL containing 0.5–1 million cells to a 96-well U- or V-bottom plate format or into FACS tubes for staining, passing them through a 40-µm mesh (*see* **Note 22**).
(a) 1 × 100 µL into a plate for surface stain (**steps 16–22**).
(b) 2 × 100 µL (unstimulated and stimulated) to a second plate (*see* **Note 23**), and include a no Brefeldin control (**steps 12**, **13**, and **21–30**).

## In vitro stimulation phase:

12 Keep the cells for the surface stain On ice, while setting up PMA/Ionomycin stimulation to induce production of cytokines of interest.
(a) PMA final concentration: [M] 20 ng/mL.
(b) Ionomycin [M] 1 µg/mL.
(c) 1000x stock Brefeldin A (final concentration [M] 3.0 µg/mL).

13 Incubate for 03:00:00 at 37 °C with [M] 5 % CO2.

## Surface staining:

14 During stimulation phase perform surface staining for extracellular markers (*see* **Note 24**).

15 If performing Zombie Yellow vital staining wash cells with 1 mL – 2 mL PBS.
Centrifuge at 400 x g, 00:05:00 (or if using plate format wash twice with 200 µL FACS buffer centrifuging for 00:02:00 at 400 x g). Resuspend in 20 µL PBS + 0.4 µL ZombieYellow for 00:15:00.

16 Add 20 µL 2.4G2 (anti-CD16/32) containing 0.2 µL MR1-6-FP tetramer (no fluorochrome conjugate) to block non-specific binding. Incubate for 00:15:00 dark, Room temperature.

17 Add surface cocktail (Table 2) using a cocktail made up in 10 µL FACS buffer.
Pipette carefully to mix. Stain for 00:20:00 – 00:30:00 at Room temperature.

18 For single color controls use splenocytes or compensation beads.

19 Wash cells *twice* with 2 mL FACS buffer, centrifuging at 400 x g, 00:05:00 (or if using plate format wash *three times* with 200 µL FACS buffer centrifuging for 00:02:00 at 400 x g ).

20 Resuspend cells in 100 µL FACS buffer (*see* **Note 25**). To enable estimation of absolute cell numbers, add a known number of calibration beads.

20.1 (a) Vortex calibration beads hard. Dilute (1:10) counting beads in PBS before using. To each sample 25 µL of these diluted beads was added, and an additional 10 µL of beads were saved to be counted with a hemocytometer, giving a count of X in a large square, i.e., X × $10^4$ beads/mL (which is X x 10 beads/µL, or X × 10 × 25 beads/sample). Typically add a total of 25,000 beads per sample.

20.2 (b) When samples have been acquired on flow cytometer, these calibration beads can be detected using their FSC/SSC profile and the absolute number of cells of interest can be estimated using the following approach. Total number of MAIT cells per sample = Number of MAIT cells counted on flow cytometer × Number of beads added/Number of beads counted/proportion of total lung cell suspension actually used for staining.

## Intracellular staining:

21 After 3-h stimulation, continue processing the cells for intracellular staining. Resuspend into FACS tube with + 1 mL PBS . Centrifuge at 400 x g, 00:05:00 . (Alternatively, if in 96-well format resuspend in 100 µL PBS , centrifuge at 400 x g, 00:02:00 and repeat.)

22 Resuspend in 20 µL PBS with 0.4 µL Zombie Yellow for 00:15:00 .

23 Add 20 µL 2.4G2 (anti CD16/32) SN containing 0.2 µL unlabeled MR1-6-FP tetramer to block non-specific tetramer staining. Incubate for 00:15:00 dark, Room temperature .

24 Add surface cocktail (Table 3) using a cocktail made up in 10 µL FACS buffer . Pipette carefully to mix. Stain for 00:20:00 – 00:30:00 at Room temperature .

25 For single color controls use splenocytes or compensation beads. These may be available from being made up earlier in the protocol.

26 Wash cells *once* with 1 mL FACS buffer, centrifuging at 400 x g, 00:05:00 (or if using plate format wash *twice* with 200 µL FACS buffer centrifuging for 00:02:00 at 400 x g).

27 Resuspend in 200 µL commercially available Fixation/Permeabilizationsolution and incubate for 00:30:00 On ice.

28 Wash with 2 mL Perm Wash (diluted 1:9 with FACS buffer). Centrifuge at 400 x g, 00:05:00 (or if using plate format wash *twice* with 200 µL Perm Wash centrifuging for 00:02:00 at 400 x g).

29 Resuspend in 50 µL Perm Wash containing intracellular cocktail (Table 4) and pipette carefully to mix. Incubate for 00:45:00 or leave to stain Overnight.

30 Wash cells with 2 mL PermWash (or if using plate format wash *twice* with 200 µL Perm Wash centrifuging for 00:02:00 at 400 x g). Resuspend cells in 100 µL FACS wash. If cells are in plate format use a multichannel pipette to transfer them to 1.2 mL “bullet” cluster tubes for acquisition, or use a plate reader attachment with the cytometer.

31 Analyze cells on flow cytometer.