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Mouse lung MAIT cell expansion and purification protocol

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DISCLAIMER

The Authors declare no competing interests.

ABSTRACT

Mucosal Associated Invariant T (MAIT) cells are unconventional T cells present abundantly in human tissues. MAIT cells interact with antigens presented by MR1, an MHC class I protein, to fight microbial infections. They also reportedly activate through cytokines to combat viruses. While MAIT cells exhibit the innate-like characteristic of rapid responses to infections, they also display adaptive-like qualities such as an effector-memory phenotype. Defining the role of MAIT cells in the immune system is essential to understand lung immune responses against bacterial infections. The following protocol outlines the expansion of MAIT cells via a cultured Salmonella Typhimurium BRD509 vaccine strain. Six days after infecting mice retro-pharyngeally with BRD509, MAIT cells were extracted from the lungs. The cells were then enriched and labeled with fluorescence for sorting. Once sorted, MAIT cells were plated and activated with anti-CD3/CD28. The cells were then analyzed with flow cytometry.

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EXTERNAL LINK

https://www.lji.org/labs/kronenberg

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MAIT cell in vivo expansion with BRD509

1 Salmonella enterica serotype Typhimurium BRD509 vaccine strain culture

6d

Work in BSL2 biosafety conditions.

1.1 Day 0: Overnight culture of Salmonella Typhimurium BRD509 strain.

10m

1. Prepare **5 mL** of LB media with **100 µg/ml** of Streptomycin.
2. Pick **-80 °C** BRD509 stock and inoculate tube.
3. Incubate **Overnight** in agitation **225 rpm, 37°C** .

1.2 Day 1: Prepare 4h culture of BRD509.

5h

1. Take **1 mL** of overnight BRD509 culture and dilute with LB media until **5 mL** .
2. Culture for an additional **04:00:00** to **06:00:00** .
3. Measure OD_{600} on spectrophotometer.
4. Calculate the amount of culture that will need to be diluted in **900 µL** of sterile PBS to get 10^6 CFU per mouse based on the following conversion table (input OD600):

A	B	C	D	E	F
A600	1		A600 of culture	0.6	
CFU/mL	600000000		CFU/mL	360000000	
			Desired CFU/mouse	2.7777777777777777	uL per mouse
	Resuspend in 900uL (30uL per mouse)			83.33333333333333	uL for 30 mice

A600 BRD509 spreadsheet

1.3 Day 1: Infection Mice

1h

1. Anesthetize the mice with isoflurane gas.
2. Administer retro-pharyngeal injections of **30 µL** of diluted BRD509 per mouse.
3. Prepare dilutions of the original dose at 10^4 and 10^5 in sterile PBS.
4. Plate dilutions on LB agar plate with Streptomycin. Count colonies next day to calculate actual dose.

MAIT cell sorting 2h

2 Prepare Lungs MAIT cells

Work in BSL2 biosafety conditions.

2.1 Day 6: Collect lungs

45m

1. Collect lungs from infected mice and process in gentleMACS C-Tube **Miltenyi Biotec Catalog #130-093-334** with **2 mL** of

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Spleen Dissociation Medium 10 x 4 mL **Stemcell Technologies Catalog #7915** using program 37C_m_LDK_1. This should take about **00:30:00** .

Cell strainer 70um

2. Filter single cell suspension through filter **Falcon Catalog #352350** on 50 mL conical tube. Mash any remaining bits of tissue through the strainer with a syringe plunger. Wash strainer with HBSS with 10% FBS until **25 mL** .

3. Centrifuge at **1500 rpm, 4°C, 00:05:00** .

4. Lyse red blood cells by adding **1 mL** of Red Blood Cell Lysis Buffer Hybri-Max **Sigma Aldrich Catalog #R7757** . Incubate at **Room temperature** for **00:05:00** .

5. Add **14 mL** of HBSS 10%FBS and centrifuge at **1500 rpm, 4°C, 00:05:00** .

6. Re-suspend cells in **2 mL** of MACS buffer.

2.2 MAIT cell enrichment

20m 30s

1. Add 50 ul of Rat Serum to sample.
2. Add the following biotin-conjugated antibodies:

A	B	C
Antigen	**Stock [ ] (mg/ml)**	**V in 2 ml (ul)**
CD11b	0.5	4
CD11c	0.5	4
Ter119	0.5	4
F4/80	0.5	4
B220	0.5	4
Ly6G	0.5	4

Biotin-conjugated antibodies

3. Incubate at **Room temperature** for **00:10:00** .

4. Vortex EasySep™ Mouse Streptavidin RapidSpheres™ Isolation Kit For processing 1 x 10^9 cells **Stemcell Technologies Catalog #19860** for **00:00:30** . Add **50 µL** of RapidSpheres to sample.

5. Mix and incubate for **00:05:00** at **Room temperature** .

6. Add **2 mL** MACS Buffer.

7. Place tube into The Big Easy EasySep™ Magnet For isolating 1 x 10^9 **Stemcell Technologies Catalog #18001** . Incubate for **00:05:00** at **Room temperature** .

8. Gently pour supernatant in a new 5 ml tube.

2.3 Fluorescent labelling

1h 35m

1. Stain cells with PE-conjugated or mouse MR1 tetramer loaded with 5-OP-RU at 1:300 dilution for **00:45:00** at **Room temperature** . Wash with PBS 2% FBS and centrifuge at **1500 rpm, 4°C, 00:05:00** .

Mouse MR1 tetramers were provided by the NIH Tetramer Core Facility: https://tetramer.yerkes.emory.edu/

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1. Stain cells with
LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation **Thermo Fisher Catalog #L34967**
at 1:500 dilution, FcBlock (2G4) at 1:500 dilution and 1:500 dilution of **1 mg/mL** Free Streptavidin in PBS.

NOTE: Make sure the PBS has no other protein content.

3. Incubate at **4 °C** for **00:15:00** . Wash with PBS 2% FBS.
4. Stain cells with the following antibodies:

A	B	C	D	E	F	G
#	Marker	Population	Channel	Host	Clone	Dilution
2	gd TCR	DUMP	PerCP-Cy5.5	Mouse	GL3	1:400
3	IgD	DUMP	PerCP-Cy5.5	Rat	11-26c.2a	1:300
4	CD11b	DUMP	PerCP-Cy5.5	Rat	M1/70	1:300
5	CD11c	DUMP	PerCP-Cy5.5	Rat	N418	1:300
6	B220	DUMP	PerCP-Cy5.5	Rat	RA36B2	1:300
7	5-OP-RU	MAIT cells	PE	-	-	1:300
8	TCR beta	T cells	APC-eF780	Mouse	H57-597	1:300
9	Live/Dead	Yellow	BV570	Rat	MEL14	1:200
10	CD45	Lymphocytes	BV785	Rat	30.F11	1:300

MAIT cell Sorting Panel

4. Incubate at **4 °C** for **00:30:00** .
5. Wash cells with PBS 2% FBS. Resuspend in **4 mL** .

3 Cell Sorting

1. Sort MAIT cells with 85 nm nozzle on low pressure. Sort into **500 µL** of sterile FBS in 5 ml FACS tubes.
2. Gate samples as following: Lymphocytes/Singlets/Live/DUMP-CD45+/Tetramer+TCRbint

You should obtain a 20-30% frequency of MAIT cells from the DUMP-CD45+ gate. Total number of MAIT cells should be between 20,000-50,000 per lung.

In vitro culture 2h

4 Day 1: MAIT cell activation with anti-CD3/CD28

4.1 Coat 48-well plate 2h

While MAIT cells are been sorted, prepare anti-CD3e + anti-CD28 coated plates.

1. Dilute anti-CD3e (2C11) to **5 µg/ml** and anti-CD28 to **2 µg/ml** in sterile PBS.
2. Add **100 µL** to each well of a 48-well plate.
3. Incubate at **37 °C** for **02:00:00** .
4. Wash plate with **200 µL** of PBS, twice.
5. remove all liquid from every well.

4.2 Plate MAIT cells 10m

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- MAIT cell culture media: RPMI 10% FBS 1X Pen/Strep 1X Pyruvate 1X HEPES 55 uM β-mercaptoethanol.

1. Wash sorted cells with MAIT cell culture media.
2. Resuspend cells in MAIT cell at 0.5 x 10^6 cells/mL in MAIT cell culture media supplemented with the following cytokines:

A	B	C	D
Cytokine	**Final [ ]**	**Stock [ ]**	**Dilution**
IL-2	10 ng/ml	20 ug/ml	1:2000
IL-7	20 ng/ml	20 ug/ml	1:1000
IL-1b	10 ng/ml	100 ug/ml	1:1000
IL-23	10 ng/ml	10 ug/ml	1:1000

Cytokines

3. Plate 500,000 cells per well.

5 Day 2: Put in fresh plate. 5m

6 Analyze MAIT cells through flow 1h 35m

1. Stain cells with PE-conjugated or mouse MR1 tetramer loaded with 5-OP-RU at 1:300 dilution for **00:45:00** at **Room temperature** . Wash with PBS 2% FBS and centrifuge at **1500 rpm, 4°C, 00:05:00** .
2. Stain cells with
   LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation **Thermo Fisher Catalog #L34967**
   at 1:500 dilution, FcBlock (2G4) at 1:500 dilution and 1:500 dilution of **1 mg/mL** Free Streptavidin in PBS.

NOTE: Make sure the PBS has no other protein content.

3. Incubate at **4 °C** for **00:15:00** . Wash with PBS 2% FBS.
4. Stain cells with the following antibodies:

A	B	C	D	E	F	G	H	I	J
#	**Marker**	**Population**	**Channel**	**Host**	**Clone**	**Dilution**			
2	gd TCR	DUMP	PerCP-Cy5.5	Mouse	GL3	1:400			
3	IgD	DUMP	PerCP-Cy5.5	Rat	11-26c.2a	1:300			
4	CD11b	DUMP	PerCP-Cy5.5	Rat	M1/70	1:300			
5	CD11c	DUMP	PerCP-Cy5.5	Rat	N418	1:300			
6	B220	DUMP	PerCP-Cy5.5	Rat	RA36B2	1:300			
7	5-OP-RU	MAIT cells	PE	-	-	1:300			
8	TCR beta	T cells	APC-eF780	Mouse	H57-597	1:300			
9	Live/Dead	Yellow	BV570	Rat	MEL14	1:200			
10	CD45	Lymphocytes	BV785	Rat	30.F11	1:300			

4. Incubate at **4 °C** for **00:30:00** .
5. Wash cells with PBS 2% FBS. Resuspend in **4 mL** .
6. Analyze cells through flow.

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