

Get to the bottom of hCD1d unlabeled staining

September 28-30th, 2021

LSR-II
HEU: NKT/MAIT-Spectral #1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf 144	HU										
Inf 148	HU										
Inf 134	Heu-L										
Inf 159	HEU-h										
NYC55	Adult										

LSR-II run

x 11

V450	BV421	CD4	1.2	19.8
V525	BV510	Aqua/D	1.500	—
V650	—	—	—	—
B530	Allophycocyanin	hCD1d	1.750	(1.5) — (1.5)
B710	PerCP-Cy5.5	CD45RA	2	22
Y590	PE	CD161	0.5	5.5
Y780	PE-Cy7	CD3	0.5	5.5
R670	Allophycocyanin	hMR1	1.200	(5) (5)
R780	APC-Cy7	CD8	1.5	(—) 16.5

11
x .8
8.8 → 9/13 hO Aqua

6.3 μl + 20.5
14.8 μl
- 6.3
7.7

10.5000
500:4500

PBS 18 μl 88
(14 μl dew)

Brought the FSC to 585 down to 585 for this one
- forgot to set stoppage gate ↓

Thawed @ 10:41, 12:00 & ~2 pm ish.

- sat in R10, washed, sat @ 4°C until used

→ not ideal, but is what it is for today!!

h/O @ 18:56 pm

- wash twice after h/O, 1.5 ml FBS-PBS & 1 ml FBS-PBS to see effect on excess RBC lipids vs hCD1d

Wash @ 19:15 ✓

Tetramer stain @ 2:47 pm ✓

Spin @ 8:31 pm ✓

Surface @ 20:48 pm → 9:08 pm

RBC lyse → 400 μl extra blood, 300 μl resl

21:08 → 21:10 so @ 21:12 ✓

Spin w/ 1 ml 5% FBS-PBS

Wash w/ 1 ml PBS after this

Done @ 9:39 pm, w/ 150 μl PFA-PBS

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Unloaded hCD1d

Inf144 (mostly dead) → smear.

Inf148 → empty "???" (red)

Inf134 → Gross whose back? (red)

Inf139 → Sexteto

N455 → Just card for some reason...

