

(reusing ref controls week & half ago)

November 22nd, 2022

# Tetramer Titrations

4400000  
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4400000  
4400000  
4400000  
4400000

| Specimen   | Status | Location | Conc | Date     | Tasks        | Volume | Ly   | Ly+Mon | Total | 3E+6        | 2E+6        |    |
|------------|--------|----------|------|----------|--------------|--------|------|--------|-------|-------------|-------------|----|
| ND0006 (1) | Adult  |          | 15E6 | 11-14-22 |              | 2      | 6.32 | 8.00   | 12.64 | 475 $\mu$ l | 316 $\mu$ l | 47 |
| (2)        | "      |          | "    | "        |              | 2      | 5.13 | 6.56   | 10.26 | 585 $\mu$ l | 389 $\mu$ l | 58 |
| (3)        | "      |          | "    | "        | more deadish | 2      | 5.47 | 7.13   | 10.94 | 548 $\mu$ l | 366 $\mu$ l | 54 |
| (4)        | "      |          | "    | "        |              | 2      | 4.71 | 6.21   | 9.42  | 637 $\mu$ l | 425 $\mu$ l | 63 |
| (5)        | "      |          | "    | "        | more deadish | 2      | 4.46 | 6.00   | 8.92  | 623 $\mu$ l | 448 $\mu$ l | 67 |

Thaw start @ 12:08pm

first samples spin @ 12:17pm

stain @ 12:50

count @ 1:02pm

Calc's @ 13:14pm done @ 1:40pm

2 ml Perm Wash  
 $\times 9$

18ml Perm Wash  
(3-27)

9  
 $\times 3$

2.7mls  
RBC lysis

800  
 $\times 8$

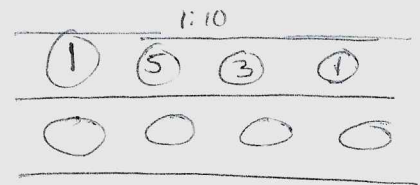
6.4mls  
2ombre  
NIR

8  
 $\times 9$

72mls  
5% FBS+PBS

Both  
loaded  
tetramers

Both  
unloaded  
tetramers



○ - Unstained for  
sample 5

$9 \times 3m = 27$  million cells

53 Million cells = 70% recovery rate

$27 + 20.1 = 47.1$  Million  
in 6 Million margin error

Mon 372 abs @ 2:08pm  
Abs + tets prepped @ 2:31

Tets

37°C inc: (48  $\mu$ l draw volume) @ 5:27  $\rightarrow$  5:57pm

spin @ 6:00pm

In @ 6:13 for 4°C  $\rightarrow$  6:43pm

RBC lysis @ 6:53pm  $\rightarrow$  spin @ 6:59pm (10min behind)

Fix Perm @ 7:11pm  $\rightarrow$  2  $\rightarrow$  31

First wash @ 7:34pm

Done @ 7:54pm



61

### Simplified Protocol

Wash with 2 ml PBS, 1400rpm, 6min

min

min

in

500m

40

2 ml Penicillin  
8 ml PBS-PBS

61



## 11/22/2022

[illegible]

| Date |        | Description         |  | Amount  |  |
|------|--------|---------------------|--|---------|--|
| 1900 | Jan 1  | Balance             |  | 100.00  |  |
| 1900 | Jan 15 | Received from A. B. |  | 50.00   |  |
| 1900 | Feb 1  | Received from C. D. |  | 25.00   |  |
| 1900 | Mar 1  | Received from E. F. |  | 75.00   |  |
| 1900 | Apr 1  | Received from G. H. |  | 100.00  |  |
| 1900 | May 1  | Received from I. J. |  | 150.00  |  |
| 1900 | Jun 1  | Received from K. L. |  | 200.00  |  |
| 1900 | Jul 1  | Received from M. N. |  | 250.00  |  |
| 1900 | Aug 1  | Received from O. P. |  | 300.00  |  |
| 1900 | Sep 1  | Received from Q. R. |  | 350.00  |  |
| 1900 | Oct 1  | Received from S. T. |  | 400.00  |  |
| 1900 | Nov 1  | Received from U. V. |  | 450.00  |  |
| 1900 | Dec 1  | Received from W. X. |  | 500.00  |  |
| 1900 | Dec 31 | Total               |  | 2500.00 |  |



November 22nd<sup>h</sup>, 2022

## Tetramer Titrations

Tetramer titration run samples @ 12:13 pm

duplicate ILT final, deleted extra parameters

(Fixed monocytes split in the V2-V7 AF signal)

Unstained run ~ 10,300 Event rate (plated 3M cells)

Monocyte FSC settings work fine for this one; not so in Monocytes if looking for debris (6800)

Going to go ahead and run CD3 & unstained cell sets & unstained beads & in this workspace.

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Unmixing Did it just crash? Sorta... (16 colors)

HMRI looks to be at lower dilution this go around (Unmixing issues in  
hCD1d similar nags)





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