

October 27-30, 2020

# HEU: PPD/TT Optimization #1

Overnight rest, 2 hours stimulation, 18 hours incubation.

1 M cells / 500  $\mu$ l

| Adult      | Location | Date | Tasks    | Volume | Ly                          | Ly+Mon              | Total  |
|------------|----------|------|----------|--------|-----------------------------|---------------------|--------|
| ND050      |          | 10E6 | 10/21/20 | 4 ml   | 1.78E4<br><del>1.78E4</del> | 2.58E4              | 7.12E6 |
|            |          |      |          | 3 ml   | 2.00E4                      | 2.51E4              | 6.00E6 |
| CVD400,282 |          | 10E6 | 10/12/20 | 4 ml   | 1.31E4                      | 1.52E4 <sup>h</sup> | 5.24E6 |
|            |          |      |          | 2 ml   | 1.68E4                      | 1.98E4              | 3.36E6 |

| Adult<br>1.0E+6 cells | Ex vivo<br>TT | Extracellular<br>x 11 |       | Intracellular<br>x 11 |            |
|-----------------------|---------------|-----------------------|-------|-----------------------|------------|
| V450 BV421            | CD69          | 1                     | 11    | CD69                  | 1 11       |
| V525 BV510            | Aqua          | 1:500                 | -     | Aqua                  | - -        |
| V670 BV650            | CD4           | 1.8                   | 19.8  | CD4                   | 0.5 5.5    |
| B530 FITC             | CD8           | 1.5                   | 16.5  | CD8                   | 0.5 5.5    |
| B710 PerCPeF710       | TNFA          | -                     | -     | TNFA                  | 3.0 33.0   |
| Y590 PE               | CD62L         | 2                     | 22    | CD62L                 | - -        |
| Y780 PE Cy7           | IFNg          | -                     | -     | IFNg                  | 0.8 8.8    |
| R670 APC              | CD3           | 1.5                   | 16.5  | CD3                   | 0.5 5.5    |
| R780 APC-Fire750      | CD45RA        | 1.5                   | 16.5  | CD45RA                | -          |
| PBS                   |               | 10.7                  | 117.7 |                       | 13.7 150.7 |

Media  
PPD: Donor  
Media  
TT Donor

PPD @ 10  $\mu$ g/ml  
C-595  
TT @ 10  $\mu$ g/ml  
C-595 - 1.78  $\mu$ l

with  
without  
with  
without  
with  
without

Excess into Spg/ml wells with priority.

$$6:6 \times 14 = \frac{6}{114}$$

ND050 190 cells/ $\mu$ l  
C4.282 120 cells/ $\mu$ l

Overnight @ 6:43pm [15 hour window]

@ 9:36am start

@ 10:01am spin

@ 10:26am 7 minutes

Running buava @ 10:35am

ND050 - 160 cells/ $\mu$ l

CVD - 130 cells/ $\mu$ l

Into incubator @ 11:21pm

St's incub @ 11:54pm  $\rightarrow$  12:37pm done

Gdgi Ply added 1:20pm ~ 7:20am stop

Intracellular @ 9:25am  $\rightarrow$  10:05am

$$\frac{800}{4.00} \sim 2M \text{ single colors}$$

$$6 \left[ \frac{1.6 M/ml \sim 500 \mu l = 800 \mu l}{= 3.75 ml} \right]$$

$$\frac{3.36}{1.6 M/ml} = 2.10 ml \text{ roughly ok}$$

Worked perfectly!

| Year | Month | Day | Time  | Location | Remarks |
|------|-------|-----|-------|----------|---------|
| 1951 | Jan   | 1   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 2   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 3   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 4   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 5   | 10:00 | 1000     | 1000    |

| Year | Month | Day | Time  | Location | Remarks |
|------|-------|-----|-------|----------|---------|
| 1951 | Jan   | 6   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 7   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 8   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 9   | 10:00 | 1000     | 1000    |
| 1951 | Jan   | 10  | 10:00 | 1000     | 1000    |

1000

1000

1000