1200 → 306g 1300 → 359g 1500 → 478g 1600 → 544g

June 29th, 2024

SFC Centrifuge Mystery #1

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
ND032			10^7	7/21/09					
ND032			10^7						

- QC run on all 3 Auroras @ 11:40am
- Thaw start @ 13:00
- 15sec w/ pellet dump for 2
- 1:07pm 2nd start
  1:11pm spin down
  1:29 pm for DNAse
  1:40 pm stain for count { 3L was not settings for FSC/SSC test 1 1:100 }
  Counts run on medium
  2:18pm fluidic shutdown count Aurora
  @ 2:23pm done

< Lunch break >

1:100 → 167 ×2 = 334
Vol eff 2x

[3:27 Aliquoting]

3:41 Down spin
3:47 writing rotor info
< 4:01 calculated >
@4:04 spin upstairs ·
4:15 back downstairs

Make sure final volume is 100µl exact

L/D @ 4:24pm → 4:36
4:30 Scls in → 4:50? spin @ 4:51
4:40 L/D wash
4:43 upstairs//
5:00 surface spin @ 5:22 down
done... @ 5:08pm 5:26 upstairs
SDBU @ 5:32 pm down
Nuclear Fix 540 → 50 ✓ → 00 ✓ → 6:10pm
6:12 pm First wash down
6:15 upstairs @ 550g
6:23 second down
6:28 upstairs se .
Pack up @ 6:34pm down
26 upst
wrong pipette...

- PE → B4 detector (CD45)
- APC → R1 detector (CD14)
- BUV805 → V16/R8 (dim! on the 3L)
- ~~BV711~~ ✓ BV750 ✓ BV786 ✓ BV421 ✓ PacBlue
- HB19
  124/264 PacBlue
  190/381 BV421] one of these for counting
  ~~750~~ 204/424 BV750

So... today:

Spin down @ ~~1000~~ 1200 rpm for 10 minutes
-(remove supernatant manually & respin later) [no sink loss]
- DNAse for 4 min
- resuspend in 2 ml
- stain 10 µl w/ 1µl CD14 & CD45 for 7 min RT
- resuspend in 500 µl

// comparison for upstairs counts //

- aliquot 100, 100, 1000 for 3 test
  - 100 + 100 for dilution test
  - 100 + 100 for dilution test

		Polysty	Polyprop
Afterwards: Aliquot for	here	O O	O O
	upstairs	O O	O O

Scls

Unst, ~~CD4~~, CD8, CD3, CD19, CD14, ~~[illegible]~~

steps / spin down / LD / 1x surface / 1x FixPerm / +2 ¿Vale?

5x

(From a similar baseline of cells / tube)

resuspended ~ equally ~ 125 µl (75/85 added w/ a couple exceptions already @ 125 µl!)
QC passed

Acquiring on medium @ 7:13 pm to match this AMs speed parameters

med 33.92 µl event rate 4,200

FSC SSC 91 342 488 for DR 2023 AB SFB
a little hanging to left SSC//

CD45 count ~ close All

$$125 \times 500 = 62500$$

70) 62500 → 900K
63000

unstained
70 µl (residual volume 10-20)
→ 534,800 All events.
533

70/500 = 125

Very little debris w/ this threshold

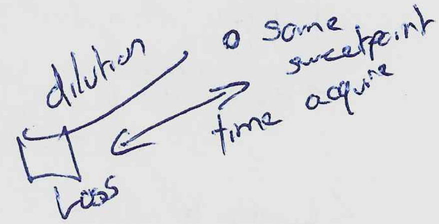
[SCs were in polypropylene & spun downstairs]

Changing hardstop to 70 µl

Samples @ 7:30 pm [4400 events @ 34.07 sec]

No apparent difference in upstairs vs. downstairs.

[3.3] → [1.0] on sip cal back



[6800 polystyrene @ 33 sec] downstairs ones

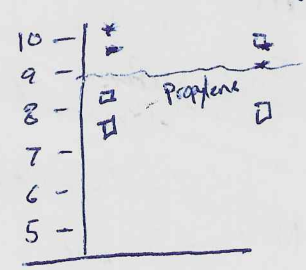
Substantial difference ... didn't make to 70 µl [64?]
← no residual volume either so...
<B still high w/ residual left>

upstairs A few 4500 (Upstairs polystyrene appear a bit lower)

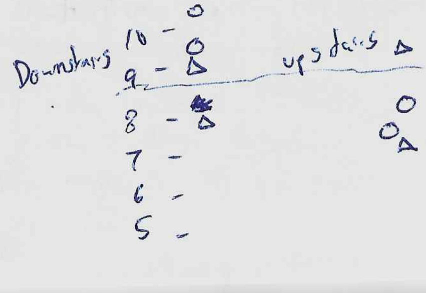
APC CD14 w/ external unstained internal OK.

[0.26 overlap APC w/ zombie]

	upstairs styrene		Downstairs styrene	
Mon	51,543	60,448	73,456	54,112
45	316,140	347,318	485,149	420,517
Beads	84,912	86,763	129,298	99,946
	451 → 850	493 → 821	687 → 1.13	573 → 935



upstairs poly		downstairs poly	
53,990	56,059	50,968	65,021
321,764	409,906	388,680	392,227
81,639	91,301	89,430	98,705
455 → 750	556 → 926	527 → 878	555 → 925



Fluidics @ 8:28 pm

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SFC Centrifuge Mystery #1

Beckman CS-6R Centrifuge

sigma aldrich .com calcs

		14 cm (g)
1000 -	976	157
1200 -	1171	225 g
1300 -	1269	265 g
1500 -	1463	352 g
1600 -	1561	401 g

~~Trau~~ ~~204~~ 145 av

5L:

546	30.18	1.8
1821	30	6.0
559	30	1.8
1820	30	6.1

Save as CellCounts3L for avoiding flowJo .wsp overlap

comparable

4L

659	2.2
1754	5.7
560	1.8
1731	5.8

3L

742	2.5
2537	8.3 ?, unclear
702	2.3

6.0 M/mL =

ST 8R rotor radius:

M-10 → 119

Tx-150 → 144

ST40 R (old we standard by)

Tx-750 = 195

1200 → 306 g
1300 → 359 g
1500 → 478 g
1600 → 544 g

20.5
1.2
× 8
9.6 μl

19.3
× 8
154.7

19.3
− 2.4
16.9
× 8
135.2

OR

48

8 + 4 = 12
× 4
48 mls

~~7.8~~
1.5
× 2
3.0 × 12 = ~~36~~

4 pcm
~~48~~ mls
36 PB
water?
di

12 mls = 3 + 9

June 29th, 2024

SFC Centrifuge Mystery #1

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