

PPD, ele agbea? 1.5 f+c

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	
CVD4.282	BCG+ done		2x 10E6	10/12/20		1.2	8.45	11.3	13.56	1.5E+6 3E+6
NY060	Adult Naive?		15E6	06/03/21		2	7.03	8.65	17.3	1.73ml -15
CVD4.282	"		15 Leu/ml	"		2	4.03	4.53*	8.06	1.5E6 372pl
NY060	"		"	"		2	6.80	8.85	13.6	220pl

Using a 12 well instead of a 6 well (cells ~ closer)

CVD $\sim 11.43 \mu\text{l} \sim 8.7 \text{ m}$

$N_{YOGO} \sim 13.5 \mu l \sim 7.4 M$

→ 2 wells

→ 3 wells (tight fit) ← droplets, don't do

Incubation start @ 9:11 pm

No more than 4 mb / well
next time

← Cells mostly happy, desert cover surface both

8/10/23 9:10 AM ← Cells mostly happy, desert cover surface.
collection start, remove excess RD into 50 ml conical
recollect w/ 5 ml pipette. ~~resor~~ add 2 ml flat warm RAPI.
wait 5 min, tap [use 1 ml next time, splitters]

10:08 stan^{for} count (CUD4282, NY060, [NY062_{rz}])

$CD \sim \frac{20}{100} \sim 10\%$ "dropped during stain, may have lost a droplet."

NYO60 ~ 10.8 μ l

Gelg. Block

5:5:95 ~ 500 μ l

$$\langle 5 \mu \rangle$$

Incubation start @ 11:26am

Golgi Mark added @ 1:48 pm

Out @ 9:11 PM ✓ Tq. 45:00

< Ab mix prepped @ 4:04 pm >

2 Non mix stopped @ 4:35pm

Ad mix rec'd done by 5:25pm

CD107a PPD SL from CVD 4282

Into spin @ 9:30 pm

AB 5

Monocytes

unsteril

CVD

NYO60

201072-5A1 PPD

Ochril
+ Gobi

+ gelb bläue
+ Sp/PPD
+ gelb. bläue
+ cD/02a

03/04/2018

\rightarrow gold
 \rightarrow gold



$\frac{1}{2} \text{P.D.} + \text{g.d.g.}$

[illegible]

+HLA-DR (sc

$(+C)16 \quad S \in \mathbb{Q}$





 15/11/20
 [NY 060]

Unit BUSIO 50's

BV510? CO45RA?

NYOGO

① CD107a ✓ = CVD4282

○ Zamben NIR ✓ ○ Zambie NIR ✓

PPD: 2052 D3Ued

h/d @ 22:00 pm (+ 3 μ l to Mon Fg3bch)

~~10:35 pm~~

→ spin w/ correct FBS's

@ 22:20 pm ✓

Mon/Ab Scs @ 22:12

10:42 pm

4°C

↓
separate washes

RT CD161 bctn next!! @ 22:33 (Mon holding w/)

Abs → 65.2 μ l

Mon → 58.3 μ l

Hct 37°C @ 10:47

→ 11:17 pm

3 min RBC lysis

@ 22:44 pm → spin @ 10:55 pm

Fix
@ 11:09-19-29

0.47% ✓✓

Done @ 11:11
50 μ l

11:17 pm Hct's done

into 2nd perm wash
@ 11:42

Hct spin @ 11:22 pm →

Post RBC lysis spin @ 00:12 am

Cold Abs in @ before 11:42 pm 25?
37 → 12:07 pm/?

AB's get
sterilized 4°C
Ser 15 min RT

Monocytes 0.47%

Done @ 00:26 am

@ 00:24 → 30

0854 900 Nov 22 099 210/00

January 3rd, 2023

PPD, ele agbea?

Fix Person @ 00:53 pm → 1:03 → 1:15 am ✓

2nd person work @ 1:23 am

55.2 p/

Intro @ 01:31 am → 2:11 am

Done @ 2:22 am

#	Single color Filter (ul)	Unmixing Ref ctrl type ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	L/D + FC blocker 3ul/rxn	HotStain 30min @37C	4	ColdStain 30min @4C	4
1	UV2		BUV395	CD18	(6.7)			0.6	2.4		
UV6			AF	AF							
UV7			BUV496								
2	UV9		BUV563	CCR2	(IS132.1D9)			0.6	2.4		
UV10											
3	UV11		BUV661	CD3CR1	(249-1)			1	4		
4	UV14		BUV737	CD19							
5	UV16		BUV805	CD14	(MSE2)			1	4		4.8
V1			BU421								
6	V3		Pacific blue	CD66b							
V5			BUV480							1.0	4
7	V7		BUV510	CD64							
8	V8		BUV570	CD16	(368)			1.2	4.8		
9	V10		BUV605	CD56	(5.1H11)					1.5	6
10	V11		BUV650	CD1c	(8-146)					1.0	4
11	V13		BUV711	FCER1a				1	4		
V14											
12	V15		BUV750	<CD4>						1.2	4.8
13	B2		BUV785	CD141						<1.2>	
B3			FLTC	CD11b						1.2	4.8
14	B4		Spark blue 550	CD3						0.4	1.6
B6			PE	CD88	(55/1)					1.2	4.8
15	B8		PE-Dazzle594	<CD3>				0.4	1.6		
16	B10		PE-Cy5	CD86	(172.2)					<0.6>	#####
17	B13		PerCP-Cy5.5	CD123					0	0.5	2
R1			PE-Vio770	HLA-DR	(RE4805)			2	8		
18	R2		Alexa Fluor 647	CD163						0.3	1.2
19	R4		APC-R720	CD40	(GHI/61)			1	4		
20	R6		Zombie NIR	L/D	(5C3)			1.0	4		
21	R7		APC/Fire 750	CD4			<1.2>500>				
21	R8		APC/Fire 810	CD38	(HI72)			1.0	4		2
			True Stain FCX					0.5	2		
			Antibody Total					11.3	45.2		
			Brilliant Stain					50	200		
			Pipette draw volume/sample					58.3		58.2	
And UNSTAINED CONTROLS !!!											

Simplified Protocol

Thaw cells, DNase, count.

Collect, count, aliquot cells 2.0E+6 Cells R10 / 5ml polystyrene tube

Bring volume up to <500> ul R10, add <>

Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min

800 ul of Live/Dead mix (1:2500) @RT for 15min

Wash 2 ml 2% PBS-FBS + EDTA, spin 1300 rpm, 8min

Add tetramers for 40 minutes at RT

Wash with 2 ml PBS, spin down 1300rpm 8min

Add HotStain mix, incubate @37C for 30 min

Wash 2 ml 2% PBS-FBS + EDTA 1400 rpm, 6min

Add ColdStain mix, incubate @4C for 30min

Add 300-500 ul 1x RBC Lysis for 3 minutes

Wash 2 ml 2% PBS-FBS + EDTA 1400 rpm, 6min

Resuspend in 100 ul 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C

Notes:

Thaw cells, DNase, count.

Thaw cells, DNase, count.

<Resuspend at 1E6/ml of R10, rest cells for <12>hrs in a 6-well plate. :
Collect, count, aliquot cells 2.0E+6 Cells/5ml polystyrene tube

Collect, count, aliquot cells 2.0E+6 Cells/ 5ml polystyrene tube

Add antigens, stim antibodies, and DuringStim antibodies in 500 μ l R10. Cap and incubate at 37°C for 2 hours.

Cap and incubate at 37°C for 2 hours

Add golgi block, and continue to incubate for <6> hours

Add golf, block, and continue to incubate for <6> hours

Wash with 2 ml PBS, spin down 1300rpm 8min

Wash with 2 ml PBS, spin down 1300rpm 8min

Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at 4°C.

Add CD161 biotin antibody for 10 minutes at 4°C.

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min

Add ColdStain mix, incubate @ 4C for 30min

Wash 2 ml 5% PBS-FBS 1400 rpm 5min

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Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

1 ml Nuclear FixPerm. Incubate @ 4C for 30m

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(vortex e)

(vortex e

First Perm Wash: 2 ml Nuc

Second Perm Wash: 2 ml Nuc

Second Perm Wash: 2 ml Nuc

Add Intracellular Stain, incubate @ RT for 40m

First Perm Wash. 2-11 Nov

2 ml Nucl

Second Perm Wash: 2 ml Nuc

[illegible]

100

cap tubes, wrap rack in foil, store at 4°C

Cap tubes, wrap rack in foil, store at 4°C

1/4/2023

[illegible]

1/4/2023

[illegible]

January 3rd, 2023

Aurora on 8:54 am

PPD, ele agbea?

Last user removed the water tube ^{II?} needle exposed.

Liz?

or did I take tube off and forget? heard it retract

January 3rd, 2023

PPD, ele agbea?

