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PBMC surface flow staining

cecilia [1], Alessandro Sette[1]

[1]La Jolla Institute for Immunology

dx.doi.org/10.17504/protocols.io.bwu9pez6

Yaqian Xu

ABSTRACT

This protocol is about Flow staining.

ATTACHMENTS

Flow staining.pdf

DOI

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PROTOCOL CITATION

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Flow staining, PBMC, staining, antibody

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OWNERSHIP HISTORY

Jul 24, 2021 Julia Rossmanith protocols.io

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PROTOCOL INTEGER ID

51841

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Day 1 - THAWING of PBMC 1h 1m 30s

1

Add **10 mL cell culture media** per donor vial being thawed to a labeled 50 ml tube.

2 Add **20 µl** per 10 ml media of Benzonase to the 50 ml tube.

3 Get as many vials of PBMC as needed.

4 Place the vials in **37 °C** water bath to thaw – approximately **00:01:30** . 1m 30s

5 Before cells are completely thawed add them to the prepared 50ml tube.

6  7m

Centrifuge tube with cells at **1200 rpm, 00:07:00** .

7 

Pour off media (cells in pellet) and resuspend in **20 mL cell culture media** .

8 

Take **20 µl** and count cells (using trypan blue).

9  7m

Centrifuge tube with cells again at **1200 rpm, 00:07:00** .<>

10 Pour off media and resuspend according to cell count to a final concentration of $10x10^6$ cells per ml with HR5 media.

A	B	C	D	E	F	G
Donor	Vials	Cells/ml ($x10^6$)	Volume (mL)	Total # PBMCs ($x10^6$)	Volume to reach $10x10^6$	% viability
	1		7			

11 

Add **100 µl** cells per well per plate (use 0.5 million instead of 1 million if necessary).

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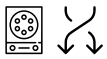
A	B	C	D	E	F	G	H	I	J	K	L	M
	1	2	3	4	5	6	7	8	9	10	11	12
A	Aliquot 1											
B												
C	Aliquot 3 unstained											
D												
E												
F												
G												
H												

12  2m

Spin plate **1400 rpm, 4°C, 00:02:00** , flick off liquid.

13  2m

Add **200 µl cold PBS buffer** , spin at **1400 rpm, 4°C, 00:02:00** , flick off liquid.

14 

Resuspend cells in **100 µl PBS with 10% FBS** (**4500 µl PBS** , **500 µl FBS**) for blocking.

15  10m

Incubate **00:10:00** at **4 °C** (or in the fridge).

16 

Add **100 µl** per well of stain mastermix (on top of the **[M]10 % FBS** so the total volume in each well becomes **200 µl**).

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A	B	C
Antibody	Amount per tube	STAIN Mastermix
CD4 APCEf780	1 µl ebio 47004942	2 µl
CD3 Alexa Fluor 700	2 µl BD 557943	4 µl
CD8 BV650	1µl Biolegend 301042	2 µl
CD19 PECy7	1 µl Tonbo 60-0199-T100	2 µl
CD14 APC	1 µl Tonbo 20-0149-T100	2 µl
CCR7 PerCPCy5.5	BioLegend 3ul 353220	6 µl
CD56 PE	2ul Ebio 8025-0567-025	4 µl
CD25 FITC	2ul (large ab box) BD 555431	4 µl
CD45RA eF450	Ebio 1 µl Ebio 48-0458-42	2 µl
Live/Dead Aqua	Ebio (stored in freezer #11) 0.2ul	0.5 µl
PBS buffer	86µl per sample	172 µl

17 30m

Incubate in dark in fridge **00:30:00** .

18 2m

Spin plate **1400 rpm, 00:02:00** , flick off liquid.

19

Wash 2x with **100 µl** per well of cold PBS Buffer.

20 Resuspend in **100 µl PBS** and transfer to FACS tubes.

Compensation controls with Beads 17m

21

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A	B	C	D	E	F
		Staining controls			
	Host	Conjugation	Bead/cells	µl/test/well	µl buffer/well
Unstained control			Beads		100
CD25	Mouse	FITC	Beads	2 µl/test	100
CD56	Mouse	PE	Beads	2 µl/test	100
CCR7	Mouse	PerCPCy5.5	Beads	2 µl/test	100
CDI9		PECy7	Beads	1 µl/test	100
CD45RA		eF450	Beads	1 µl/test	100
CD19 instead of live/dead	Mouse	V500	Beads	1 µl/test	100
CD8	Mouse	BV650	Beads	1 µl/test	100
CD14	Mouse	APC	Beads	1 µl/test	100
CD3	Mouse	AF700	Beads	2 µl/test	100
CD4	Mouse	APCEf780	Beads	1 µl/test	100

22 Add 1 drop beads (**ebio** Ultra comp beads).

23 2m

Spin down **1400 rpm, 4°C, 00:02:00** .

24

Add **100 µl PBS buffer and designated antibody** .

25 15m

Incubate in the dark for **00:15:00** in fridge.

26

Wash twice with cold PBS buffer.

27

Resuspend to **100 µl PBS buffer** and add to FACS tubes.

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