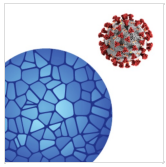

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COVID Blood Processing for scRNAseq

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1 Works for me dx.doi.org/10.17504/protocols.io.bjm6kk9e

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ABSTRACT

This protocol describes the isolation of lymphocytes and pan-mononuclear cells from human whole blood for scRNAseq analysis.

ATTACHMENTS

Columbia_Wells_COVID_Blood_RosetteSep_Processing_for_scRNAseq.pdf

DOI

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PROTOCOL CITATION

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KEYWORDS

SARS-CoV-2, COVID-19, lymphocytes, isolation, pan-mononuclear cells, human whole blood, scRNAseq

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OWNERSHIP HISTORY

Aug 12, 2020 Julia Rossmanith protocols.io

Sep 02, 2020 Peter Sims

PROTOCOL INTEGER ID

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MATERIALS

NAME	CATALOG #	VENDOR
Gibco™ DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific
Penicillin-Streptomycin-Glutamine (100X)	10378016	Thermo Fisher
UltraPure™ 0.5M EDTA, pH 8.0	15575020	Thermo Fisher

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NAME	CATALOG #	VENDOR
Thermo Scientific™ Nunc™ 50mL Conical Sterile Polypropylene Centrifuge Tubes	12-565-271	Fisher Scientific
5mL Falcon™ Round-Bottom Polypropylene Test Tubes	14-959-11A	Fisher Scientific
Biotin anti-human CD235ab Antibody	306618	BioLegend
Biotin anti-human CD66b Antibody	305120	BioLegend
BioMag® Plus Streptavidin	BP628	Bangs Laboratories
Corning™ Externally Threaded Cryogenic Vials	09-761-71	Fisher Scientific
CryoStor CS10 100ML	NC9930384	Fisher Scientific
Gibco™ Fetal Bovine Serum qualified Australia	10-099-141	Fisher Scientific
Ficoll-Paque™ PLUS Media	45-001-749	Fisher Scientific
Human TruStain FcX™	422302	BioLegend
NC-Slide A8™ box with 25 Slides	942-0003	Chemometec
Solution 13 AO – DAPI	910-3013	Chemometec
Dead Cell Removal Kit	130-090-101	Miltenyi Biotec
MS Columns	130-042-201	Miltenyi Biotec
RosetteSep™ Human Granulocyte Depletion Cocktail	15624	Stemcell Technologies

MATERIALS TEXT

Equipment

- Centrifuge
- Cell Counter - NC-3000
- EasyEights™ EasySep™ Magnet (Stemcell Technologies, Cat. No.: 18103)

EasyEights™ EasySep™ Magnet
Magnet for column-free immunomagnetic separation
EasySep **18103**

- MACS Multistand (Miltenyi, Cat. No.: 130-108-934)

MACS MultiStand
Separator for magnetic cell separation
MACS **130-108-934**

EQUIPMENT

NAME	CATALOG #	VENDOR
EasyEights™ EasySep™ Magnet	18103	Stemcell Technologies
MACS MultiStand	130-108-934	Miltenyi Biotec

SAFETY WARNINGS

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

Biosafety Notes

- All materials required for sample processing are to be prepared in the biosafety cabinet before handling of blood samples.

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- All blood sample manipulation takes place in a biosafety cabinet unless specifically stated.
- All centrifugation steps must take place in capped containers. Upon completion of a centrifugation step, return the capped container to the biosafety cabinet, remove all tubes, and spray and wipe them with >70% ethanol before continuing to the next step.

Preparing Buffer

1

Create the following **DPBS Solution-EDTA** in a bottle of DPBS by using the table below:

Component	*Volume (mL)*	*Starting Conc.*	*Final Conc.*
DPBS	474	-	
FBS	25	100%	5%
EDTA	1	0.5 M	1 mM

Table 1.

Preparation of Blood

2 Place sample box into biosafety cabinet.

3 Remove samples from the box and from the containment bags, discard bags, spray sample containers with >70% ethanol and wipe down.

4 Record the total volume of whole blood to be processed in mL.

5

Spin the whole blood **400 x g, 20°C, 00:10:00** in the anti-coagulant tubes and remove the plasma layer to cryovials. Be sure to not remove any red blood cells with the plasma. **Add the same volume to all cryovials**.

Record number of vials: _____ and the volume per vial _____ mL.

NOTE: Ensure that cryovials are decomtaminated prior to removal from the biosafety cabinet.

6

Add the same volume of **DPBS Solution-EDTA** as removed plasma back to the blood tube. Pipette to mix.

RosetteSep and Ficoll-Paque

7

Add **50 µl RosetteSep Cocktail/1mL of blood** directly to the blood tube. Pipette to mix.

8

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Inclubate samples at **Room temperature** for **00:20:00** .

9

Transfer sample to a 50 mL tube and dilute *up to* **25 mL** with DPBS Solution-EDTA solution.

10

Aliquot **15 mL Ficoll-Paque Media PLUS** to a 50 mL tube.

11

Using the **slow** setting on the pipette gun, **gently** layer the blood/DPBS Solution-EDTA mixture on top of the **15 mL Ficoll-Paque Media PLUS** . **Take extra care not to disturb the blood-ficoll interface while layering. Disturbing the interface excessively prevents the mononuclear cells from becoming a clean layer.**

12

Spin for **1200 x g, 20°C, 00:20:00** with **no brake**, 4 acceleration.

NOTE: Centrifuge should be pre-warmed to **20 °C** .

13

Remove the mononuclear cell layer from each tube and transfer to a new 50 mL tube. **Take extra care to avoid pulling cells from the ficoll layer (underneath the mononuclear cell layer) as this typically contains a lot of granulocytes.** Pulling from the plasma layer is not an issue.

14

Top PBMC with **cold** DPBS Solution-EDTA *to* **40 mL** (ensure at least 2-3 volumes are added) and centrifuge the cell suspension(s) for **400 x g, 4°C, 00:10:00** .

15

(Platelet Spin) Discard the supernatant, top tube to **40 mL** with **cold** DPBS Solution-EDTA, and centrifuge the cell suspension for **120 x g, 4°C, 00:10:00** .

16

Remove the supernatant (**caution: pellet may be loose**), and resuspend the cell pellet in

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4 mL Dulbecco's Phosphate Buffered Saline (DPBS) .

Cell Counting of COVID Samples

17

Add **0.05 mL sample** , **0.05 mL DPBS** , and **0.005 mL Solution 13** to a 1.5 mL centrifuge tube, incubate for **00:02:00** at **Room temperature** .

18

Add **0.1 mL BD Cytofix Fixation Buffer** to the samples and incubate **00:30:00** , **4 °C** , and protect from light.

19

Aliquot **0.01 mL sample** to the well of a NC-Slide A8 and count on the NC-3000.

Record number and viability below, calculate total cells:

cell number: _____cells/mL, _____% viable

Division of Sample for Analysis and Freeze-down

20

Aliquot up to 2 x 10^7 cells to a 5 mL Falcon Round-Bottom tube and place **On ice** for subsequent sample clean-up (next section).

21 Freeze down up to 1 x 10^8 cells in approximately 1 x 10^7 aliquots (**1 mL** each) using Cryostor CS10 Medium, a Mr. Frosty, and a **-80 °C** freezer.

Record the number of vials frozen: _____ and the cells per cryovial frozen: _____.

Sample Clean Up for scRNAseq – CD66b and CD235ab removal

22

Centrifuge the single cell suspension for **400 x g, 4°C, 00:05:00** .

23

Discard the supernatant and resuspend the cell pellet in **50 µl DPBS Solution-EDTA** .

24

Add **10 µl Human TruStain FcX** to the single cell suspension and incubate for **00:10:00** , **4 °C** .

25

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Add **10 µl biotinylated anti-CD66b and biotinylated anti-CD235ab** to the sample and incubate for **00:30:00** , **4 °C** .

26

While the single cell suspension is incubating add **0.2 mL BioMag Plus Streptavidin Beads** to a 5 mL Falcon Round-Bottom tube.

27

Add **2 mL DPBS Solution-EDTA** to the BioMag Plus Streptavidin Beads and place on a magnet for **00:05:00** .

28

Remove all the supernatant from the BioMag Plus Streptavidin Beads, remove from the magnet and resuspend the beads in **0.1 mL DPBS Solution-EDTA** .

29

Once step 25 (**go to step #25**) is complete, add **3 mL DPBS Solution-EDTA** to the single cell suspension and centrifuge for **400 x g, 4°C, 00:05:00** .

30

Resuspend the single cell suspension in the BioMag Plus Streptavidin Beads from step 28 (**go to step #28**), and incubate at **Room temperature** for **00:05:00** .

31

Add **3 mL DPBS** to the tube and place on a magnet for **00:05:00** .

32

Remove supernatant from tube and transfer to a separate 5 mL Falcon Round Bottom tube.

Sample Clean Up for scRNAseq – Dead Cell Removal

33

Centrifuge the single cell suspension for **400 x g, 4°C, 00:05:00** , and discard supernatant.

34

Resuspend cell pellet in **0.1 mL Dead Cell Removal Microbeads** , mix well, incubate, **Room temperature** ,

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00:15:00 .

35

While the cell suspension is incubating, place an MS Column onto the MACS Multistand and rinse with **0.5 mL 1x Binding Buffer Solution** .

36

Post incubation, apply cell suspension to the MS Column and capture the flow through in a 5 mL Falcon Round Bottom tube.

37

Rinse with **1.5 mL 1x Binding Buffer** and capture in the same tube.

38

Centrifuge the single cell suspension for **400 x g, 4°C, 00:05:00** , and discard supernatant.

39

Resuspend cell pellet in **0.5 mL DPBS** , and count cells.

Cell Counting of COVID Samples (10x)

40

Add **0.05 mL sample** , **0.05 mL DPBS** , and **0.005 mL Solution 13** to a 1.5 mL centrifuge tube, incubate for **00:02:00** at **Room temperature** .

41

Add **0.1 mL BD Cytofix Fixation Buffer** to the samples and incubate **00:30:00** , **Room temperature** , and protect from light.

42

Aliquot **0.01 mL sample** to the well of a NC-Slide A8 and count on the NC-3000.

43

Record number and viability below, calculate total cells:

cell number: _____cells/mL, _____% viable

10X Encapsulation

44 Follow the appropriate 10X protocol (Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide – Rev D) for

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encapsulation of cells from the airway sample.

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