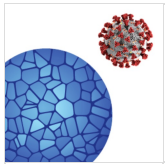

Sep 17, 2020

COVID Airway Processing for scRNAseq

Peter Szabo[1], Steven Wells[1], Peter A. Sims[1], Donna Farber[1]

[1]Columbia University

1 Works for me dx.doi.org/10.17504/protocols.io.bjj8kkrw

Peter Sims

ABSTRACT

This protocol describes the isolation of lymphocytes and pan-mononuclear cells from airway samples.

ATTACHMENTS

Columbia_Wells_COVID_Airway_Ficoll_Processing_for_scRNAseq.pdf

DOI

dx.doi.org/10.17504/protocols.io.bjj8kkrw

PROTOCOL CITATION

Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber 2020. COVID Airway Processing for scRNAseq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

KEYWORDS

SARS-CoV-2, COVID-19, lymphocytes, isolation, pan-mononuclear cells

CREATED

Aug 11, 2020

LAST MODIFIED

Sep 17, 2020

OWNERSHIP HISTORY

Aug 11, 2020 Julia Rossmanith protocols.io

Sep 02, 2020 Peter Sims

PROTOCOL INTEGER ID

40288

MATERIALS

NAME	CATALOG #	VENDOR
Gibco™ DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific
Penicillin-Streptomycin-Glutamine (100X)	10378016	Thermo Fisher
UltraPure™ 0.5 M EDTA pH 8.0	15575020	Thermo Fisher Scientific
Thermo Scientific™ Nunc™ 50mL Conical Sterile Polypropylene Centrifuge Tubes	12-565-271	Fisher Scientific

NAME	CATALOG #	VENDOR
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
Gibco™ IMDM (Iscoves Modified Dulbeccos Medium)	12-440-053	Fisher Scientific
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

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 Airway samples.
- All Airway 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

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

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 and Media

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.

2

Create **IMDM Layering Media** in a bottle of IMDM.

Component	*Volume (mL)*	*Starting Conc.*	*Final Conc.*
IMDM	470	-	
FBS	25	100%	5%
EDTA	5	100%	1%

Table 2.

Preparation of Sample

3 Place sample box into biosafety cabinet.

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

NOTE: If sputum traps come with tubing attached flush tubing with **1 mL** - **2 mL** (or more) before proceeding to optimize yield.

5 Record the total volume of Airway sample to be processed in mL.

6

Transfer the Airway Sample to a 50 mL tube, add **4 mL DPBS** to the airway sample.

7

Spin the Airway for **400 x g, 20°C, 00:10:00** , remove and save four aliquots of the supernatant in cryovials. **Add the same volume to all cryovials.**

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

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

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

8

Add **IMDM Layering Media** to the Airway sample to bring the *total volume* to **25 mL** .

9

Add **40 µl Benzonase** to the sample, pipette up and down vigorously to mix and dissociate airway pellet, incubate at **Room temperature** for **00:30:00** .

10

Add **750 µl 0.5M EDTA** , and filter sample through a *100 µM* filter.

Ficoll-Paque

11

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

12

Using the **slow** setting on the pipette gun, **gently** layer the airway/IMDM mixture on top of the **15 mL Ficoll-Paque Media PLUS** .

Take extra care not to disturb the interface while layering. Disturbing the interface excessively prevents the mononuclear cells from becoming a clean layer.

13

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

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

14

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.

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

15

Top MNC 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** .

16

(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** .

17

Remove the supernatant (**caution: pellet may be loose**), and re-suspend the cell pellet in **4.5 mL Dulbecco's Phosphate Buffered Saline (DPBS)** (final volume should be about **5 mL**).

Cell Counting of COVID Samples

18

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** .

19

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

20

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 scRNAseq Analysis and Freeze-down

21

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).

22 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: _____.

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

23

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

24

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

25

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

26

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

27

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

28

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

29

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** .

30

Once step 26 (**go to step #26**) is complete, add **3 mL DPBS Solution-EDTA** to the single cell suspension and centrifuge for **400 x g, 4°C, 00:05:00** .

31

Resuspend the single cell suspension in the BioMag Plus Streptavidin Beads from step 29 (**go to step #29**), and incubate at **Room temperature** for **00:05:00** .

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

32 

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

33 

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

Sample Clean Up for scRNAseq – Dead Cell Removal

34 

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

35 

Resuspend cell pellet in **0.1 mL Dead Cell Removal Microbeads** , mix well, incubate, **Room temperature** , **00:15:00** .

36 

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

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

38 

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

39 

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

40 

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

Cell Counting of COVID Samples (10x)

41 

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** .

42 

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw

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

43 

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

44 

Record number and viability below, calculate total cells:

cell number: _____cells/mL, _____% viable

10X Encapsulation

45 Follow the appropriate 10X protocol (Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide – Rev D) for encapsulation of cells from the airway sample.

Citation: Peter Szabo, Steven Wells, Peter A. Sims, Donna Farber (09/17/2020). COVID Airway Processing for scRNAseq. https://dx.doi.org/10.17504/protocols.io.bjj8kkrw