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COVID Blood Processing for scRNAseq

In 1 collection

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Human Cell Atlas Method Development Community

Coronavirus Method Development Community

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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](#)

MATERIALS

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- ✕ Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #14190144**
- ✕ Penicillin-Streptomycin-Glutamine (100X) **Thermo Fisher Catalog #10378016**
- ✕ UltraPure™ 0.5M EDTA, pH 8.0 **Thermo Fisher Catalog #15575020**
- ✕ Thermo Scientific™ Nunc™ 50mL Conical Sterile Polypropylene Centrifuge Tubes **Fisher Scientific Catalog #12-565-271**
- ✕ 5mL Falcon™ Round-Bottom Polypropylene Test Tubes **Fisher Scientific Catalog #14-959-11A**
- ✕ Biotin anti-human CD235ab Antibody **BioLegend Catalog #306618**
- ✕ Biotin anti-human CD66b Antibody **BioLegend Catalog #305120**
- ✕ BioMag® Plus Streptavidin **Bangs Laboratories Catalog #BP628**
- ✕ Corning™ Externally Threaded Cryogenic Vials **Fisher Scientific Catalog #09-761-71**
- ✕ CryoStor CS10 100ML **Fisher Scientific Catalog #NC9930384**
- ✕ Gibco™ Fetal Bovine Serum qualified Australia **Fisher Scientific Catalog #10-099-141**
- ✕ Ficoll-Paque™ PLUS Media **Fisher Scientific Catalog #45-001-749**
- ✕ Human TruStain FcX™ **BioLegend Catalog #422302**
- ✕ NC-Slide A8™ box with 25 Slides **Chemometec Catalog #942-0003**
- ✕ Solution 13 AO – DAPI **Chemometec Catalog #910-3013**
- ✕ Dead Cell Removal Kit **Miltenyi Biotec Catalog #130-090-101**
- ✕ MS Columns **Miltenyi Biotec Catalog #130-042-201**
- ✕ RosetteSep™ Human Granulocyte Depletion Cocktail **Stemcell Technologies Catalog #15624**

Equipment

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

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bjm6kk9e

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We use this protocol and it's working

Created: Aug 12, 2020

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PROTOCOL integer ID:
40350

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

Equipment

EasyEights™ EasySep™ Magnet

Magnet for column-free immunomagnetic separation

EasySep

18103

<https://www.stemcell.com/easyeights-easysep-magnet.html>

NAME

TYPE

BRAND

SKU

LINK

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

Equipment

MACS MultiStand

Separator for magnetic cell separation

MACS

130-108-934

<https://www.miltenyibiotec.com/DE-en/products/macs-multistand.html#130-108-934>

NAME

TYPE

BRAND

SKU

LINK

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.
- 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

NOTE: Ensure that cryovials are decontaminated 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 Incubate 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

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 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 2×10^7 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 1×10^8 cells in approximately 1×10^7 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 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, 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 Cytfix 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 encapsulation of cells from the airway sample.