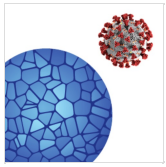

Oct 30, 2020

CITE-seq for PBMCs

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bjhmkj46

Human Cell Atlas Method Development Community | Coronavirus Method Development Community

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ABSTRACT

This is a protocol for CITE-seq for PBMCs. It includes the steps for Thawing samples and Antibody Staining.

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KEYWORDS

CITE-sequencing, PBMCs, COVID

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GUIDELINES

For antibody staining: The aim is to collect a total of ~7,000 cells per patient. For this, **25,000 cells of the pool** of 2 samples will be loaded into one channel of the 10x Chromium.

MATERIALS TEXT

MATERIALS

Flowmi cell strainer **Contributed by users Catalog #H13680-0040**

Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #422301, 422302**

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- RPMI
- 10% FBS
- water bath
- 15 ml tube
- 70% EtOH
- Centrifuge
- Class II MSC
- RBC lysis buffer
- FACS buffer
- Ficoll
- Trypan blue
- CD3+
- Low binding tubes
- MS magnetic column
- CITE-seq Antibody mix
- PBS
- 0.04 % BSA

SAFETY WARNINGS

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

ABSTRACT

This is a protocol for CITE-seq for PBMCs. It includes the steps for Thawing samples and Antibody Staining.

Thawing Samples

1 Warm up 10x the volume of thawing medium (RPMI + 10% FBS).

2 Thaw frozen PBMCs in **37 °C water bath** .

3 

Transfer sample into a 15 ml tube.

4 

Add 10x volume of thawing medium dropwise into tube.

5 Spray down with 70% EtOH.

6 

Centrifuge **500 rcf, 00:05:00** .

7 Remove the sealed bucket from the centrifuge and place into the Class II MSC.

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8 Discard supernatant.

9 Resuspend in **1 mL RBC lysis buffer** for **00:03:00** to remove erytrocytes.

10 Add **9 ml FACs buffer** .

11 

Centrifuge at **400 rcf, 00:05:00** .

12 Remove supernatant.

13 Resuspend the cell pellet in **500 µl FACS buffer** .

Equal volume for all patients.

14 Perform ficoll to eliminate dead cells.

14.1 

Add **800 µl Ficoll** and **500 µl cell suspension** to a centrifuge conical tube.

14.2 

Centrifuge at **10000 rcf, 00:00:10** .

14.3 

Swap the tube and centrifuge again at **10000 rcf, 00:00:30** .

15 Recover live cells.

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16

Wash with **1 mL FACS buffer** .

17

Centrifuge and discard supernatant.

18 Resuspend in **250 mL FACS buffer** .

19

Count cells using trypan blue.

One fraction of these PBMCs will be used directly and another fraction will be used for CD3+ cells depletion.

19.1 With the **first fraction** of PBMCs: pool 125,000 of live PBMCs from each patient (2 patients) in low binding tubes; 250,000 cells in total.

IMPORTANT: Keep the cells On ice all times!!

20 Cells should be pooled in a final volume of exactly **100 µl** .

20.1

Centrifuge and remove **87.5 µl supernatant** . Final volume **12.5 µl** .

21 Proceed with CD3 negative cells isolation using the **second fraction** of PBMCs:

21.1 Use 2x10^6 of the PBMCs (**200 µl cell suspension** + **20 µl anti-CD3 magnetic beads**).

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21.2

Incubate **00:15:00** at **4 °C** (not ice).

21.3 Resuspend in **1 mL FACS buffer** .

21.4

Centrifuge and remove supernatant.

21.5 Resuspend in **500 µl FACS buffer** .

21.6

Pass the cells through a MS magnetic column with 3 washes of **500 µl** .

21.7

Centrifuge the eluted fraction and resuspend in **200 µl FACS buffer** .

22 Count CD3 negative cells using trypan blue.

23 Pool 125,000 of CD3 negative cells from each patient (2 patients) in low binding tubes = 250,000 cells in total

IMPORTANT: Keep the cells on ice at all times!!

24 Cells should be pooled in a final volume of exactly **100 µl** .

25

Centrifuge and remove **87.5 µl supernatant** .

Final volume **12.5 µl** .

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Antibody Staining

26 Prepare CITE-seq Antibody mix:

26.1 

Use one aliquot of AB pool per sample; centrifuge at **14000 rcf, 4°C, 00:01:00** .

26.2 

Add **50 µl FACS buffer** to reconstitute.

26.3 Vortex.

26.4 

Incubate in the dark for **00:05:00** to ensure the antibodies are completely resuspended.

26.5 

Centrifuge **14000 rcf, 4°C, 00:10:00** .

27 

During the centrifugation, add **1.25 µl Human TruStain FcX™ Fc Blocking Reagent** per tube.

28 

Incubate **00:10:00** at **4 °C** .

29 

Add **12.5 µl CITE-seq AB mix** to FC blocked samples, taking care to avoid any pellet (if one exists).

30 

Incubate for **00:30:00** at **4 °C** in dark.

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31 

Add **1 mL FACS buffer** .

32 Place tubes in centrifuge bucket inside the Class II MSC and close the lid.

33 

Centrifuge the samples at **500 rcf, 4°C, 00:05:00** (swing bucket centrifuge).

34 Discard the supernatant.

35 

Repeat wash steps two more times (total of 3 washes). **go to step #31**

36 Resuspend cells in **400 µl 1x PBS + 0.04 % BSA** .

37 Filter cells through 40 µm strainers (Flowmi Cell Strainer).

38 Pass an extra volume of **400 µl** through the same flowmi strainer to recover the remaining cells.

39 

Centrifuge the sample at **500 rcf, 4°C, 00:05:00** and remove **700 µl supernatant** .

Final volume will be around **100 µl** .

40 

Count cells using Trypan blue.

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41 

Take **5 µl cell suspension** + **10 µl Trypan blue** (final dilution 1:3).

42 Proceed immediately to the 10x Genomics Single Cell protocol.

NB: The aim to is collect a total of ~7,000 cells per patient. For this, **25,000 cells of the pool** of 2 samples will be loaded into one channel of the 10x Chromium.

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