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Copy of Cell preparation for scRNA-Seq from diluted bodily fluids

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1 *Works for me* This protocol is published without a DOI.

Human Cell Atlas Method Development Community NCIHTAN 1 more workspace

Linas Mazutis

ABSTRACT

Efficient isolation of cells from complex bodily fluids is a crucial step for many biological and biomedical applications. Yet, it can become a particularly challenging when only a small fraction of cells is dispersed in a large volume of fluid, e.g. ~ 1000-10000 cells / 10 mL. In such cases a conventional wisdom would suggest the use of canonical 15 or 50 ml tubes and centrifugation forces at 500-800g to pellet the cells. However, hard centrifugation can damage the cells and rupture their membrane, while at lower centrifugation speeds the sedimentation rates are slow and might be insufficient to form a cell pellet. Moreover, intrinsic variability of cells in terms of size (6-20 µm) and density (1.06-1.29g/mL) may reduce the pelleting and recovery yields.

When using swinging bucket rotors and large sample volumes (> 5 mL) the sample sedimentation run times can become very long due to the increased sedimentation path length (distance that cells need to travel to reach bottom of the tube). On another hand, in fixed angle rotors the cells will travel only a short distance before hitting the wall of the tube and as a result will migrate down the wall forming a long trail until reaching the bottom of the tube, which will preclude formation of a tight pellet. Overall, when working with fragile cell samples preference should be given to lower centrifugal forces and shorter centrifugation times. These two requirements can be fulfilled by using smaller tubes as the shorter sedimentation path length will shorten the distance that cells need to travel to reach bottom of the tube.

Given these considerations this protocol describes the isolation of viable primary cells from diluted suspensions of bodily fluids. The protocol exemplifies the isolation of cells (immune and cancer cells) from cerebrospinal fluid where the concentration of cells is in the order of 0-20 cells / µl.

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MATERIALS TEXT

Hemocytometer **Thermo Fisher Scientific Catalog #A25750**

Microcentrifuge tubes (1.5 ml Protein LoBind tubes) **Eppendorf Catalog #022431081**

Phosphate Buffered Saline **Gibco, ThermoFisher Catalog #10010023**

Trypan Blue Solution 0.4% **Thermo Fisher Scientific Catalog #15250061**

10% (w/v) BSA filtered through 0.2 µm membrane **Sigma Catalog #A7906-100G**

Nuclease-free Water **Thermo Fisher Scientific Catalog #AM9937**

Falcon, conical centrifuge tubes **Corning Catalog #352070**

Chromium Single Cell 3 GEM Library & Gel Bead Kit v3 **10x Genomics Catalog #1000075**

Chromium Chip B Single Cell Kit **Contributed by users Catalog #1000073**

Chromium i7 Multiplex Kit **Contributed by users Catalog #120262**

Countess II
Cell counter
Thermo Fisher Scientific AMQAX1000

Swinging bucket cooling centrifuge
Centrifuge
Fisher Scientific SorvallTM LegendTM X1 Centrifuge Series

Ice bucket
. .

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Efficient isolation of cells from complex bodily fluids is a crucial step for many biological and biomedical applications. Yet, it can become a particularly challenging when only a small fraction of cells is dispersed in a large volume of fluid, e.g. ~ 1000-10000 cells / 10 mL. In such cases a conventional wisdom would suggest the use of canonical 15 or 50 ml tubes and centrifugation forces at 500-800g to pellet the cells. However, hard centrifugation can damage the cells and rupture their membrane, while at lower centrifugation speeds the sedimentation rates are slow and might be insufficient to form a cell pellet. Moreover, intrinsic variability of cells in terms of size (6-20 µm) and density (1.06-1.29g/mL) may reduce the pelleting and recovery yields.

When using swinging bucket rotors and large sample volumes (> 5 mL) the sample sedimentation run times can become very long due to the increased sedimentation path length (distance that cells need to travel to reach bottom of the tube). On another hand, in fixed angle rotors the cells will travel only a short distance before hitting the wall of the tube and as a result will migrate down the wall forming a long trail until reaching the bottom of the tube, which will preclude formation of a tight pellet. Overall, when working with fragile cell samples preference should be given to lower centrifugal forces and shorter centrifugation times. These two requirements can be fulfilled by using smaller tubes as the shorter sedimentation path length will shorten the distance that cells need to travel to reach bottom of the tube.

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Sample retrieval 1h

1 1h

Collect the bodily fluid following the protocol of choice (e.g. retrieval of cerebrospinal fluid).
Typically one could expect to have 3-5 ml of starting fluid.

Concentrating cells 22m

2 7m

- Divide the fluid from step #1 to microcentrifuge tubes at 0.5 ml per tube.

Note: When working with large volume >5 ml it may be inconvenient to handle multiple tubes. A fluid volume per single tube can be increased to 0.75-1.0 ml.

- Transfer the tubes to a swinging bucket centrifuge and spin at 300 g for 5 min at 4 ºC with breaks off. Once centrifugation is complete the cell pellet will form at the bottom of the tube, but it may be invisible to the eye.
- Place tubes on ice.

3 5m

- Carefully aspirate the supernatant from each tube leaving 50 µl on top of cell pellet.
- Gently disperse the cell pellet in each tube by slowly pipetting 5-times using 200 µl volume pipette.
- Combine the cell suspensions into one microcentrifuge tube.
- Add ice-cold 1X PBS buffer supplemented with 1% BSA to the final volume of 1 ml.

4 7m

- Divide 1 ml suspension into two tubes, each equal volume (0.5 ml).
- Centrifuge the tubes at 300 g for 5 min at 4 ºC in a swinging bucket centrifuge with breaks off.
- Place microcentrifuge tubes on ice.

5 3m

- Carefully aspirate the supernatant in each tube leaving 20-30 µl on top of pellet.
- Disperse the cell pellet in each tube by slowly pipetting 10-times using 20 µl volume pipette.
- Combine the cell suspensions into one microcentrifuge tube.

Counting cells 5m

6 5m

- Count the cells on a hematocytometer by mixing 5 µl of cell suspension with 5 µl of 0.4% Trypan Blue dye:
- If the cell count is higher than 600 cells / 1 µl proceed to scRNA-Seq step.
- If the cell count is lower than 600 cells / 1 µl then centrifuge cells again at 300g for 5 min at 4 ºC in a swinging bucket centrifuge with breaks off and discard the requirement supernatant volume to concentrate the cells. For example, if the starting volume is 100 µl one may choose to discard 70 µl and resuspend cell pellet in the remaining 30 µl of supernatant buffer (e.g. 1X PBS + 1% BSA).

Barcoding cells for scRNA-Seq 20m

7 20m

- Follow 10X genomics protocol when using Chromium instrument 10X genomics protocol (v3.1 Chemistry)
- Follow inDrops protocol when using home-built inDrops plaform inDrops protocol