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CGAP Human Lung Dissociation - Tissue Stability Study

Forked from CGAP Human Lung Dissociation

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Human Cell Atlas Method Development Community

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Material	Quantity	Supplier Info
DMEM	50ml	Lonza (LZBE12-614F)
FBS	5ml	Sigma (F7524-50ML)
Collagenase (10mg/mL stock) dilute in nuclease free water	500µl	Sigma Aldrich (C5138-25MG)
DNAse I (10mg/mL stock)	50µl	Sigma Aldrich (000000011284932001)
100mm Petri Dish	1	Corning (430591)
Forceps	2	ThermoFisher UK Ltd (15232290)
Scalpel	1	Swann-Morton Ltd (0507)
100uM Cell Strainers	2	ThermoFisher UK Ltd (15380801)
50ml Falcon Tubes	2	Falcon (352098)
2.0ml Syringe	1	ThermoFisher UK Ltd. (10673555)
70µm Cell Strainer	1	ThermoFisher UK Ltd. (15370801)
15ml Falcon Tubes	3	Falcon (352097)
Cold PBS	50ml	GIBCO (14190-144)
Red Cell Lysis Buffer	2ml	Life Technologies Ltd. (00-4333-57)
0.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.035)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)

2 Note that this protocol was originally developed for dissociation of lung airway tissue to extract immune cells. For the Tissue Stability Study we are using it on parenchyma tissue - the digestion time for this can be reduced to 30min. We found that this protocol is indeed biased towards extracting immune cells.
We are testing a different dissociation protocol that appears to be less immune-biased, but is still in development: dx.doi.org/10.17504/protocols.io.39ygr7w

3 Cut lung into 1cm x 1cm sections and ensure weight is 0.2-0.5g.

4 Transfer the piece of tissue to a 10cm petri dish and add ~250µl Digestion Medium to cover it. (Digestion medium = DMEM with 1mg/ml collagenase D and 0.1mg/ml DNase I).

5 Using two scalpels, chop the piece as finely as possible.

6 Add ~2ml of Digestion Media and transfer the mashed tissue to a 15ml falcon tube using a 5ml stripette.

7 Wash the dish with 2 x 1ml of Digestion Medium, transferring it to the 15ml falcon tube with the tissue.

8 Transfer it to an incubator at 37°C for 1 hour on a rocker.
(Note softer tissue from parenchyma e.g. lower left lobe can be left for 30 minutes. However for Tissue Stability Study the longer 1h digestion was used as airway dissociation was being tested in parallel for some donors).

9 Collect the sample and filter the cells through a 100µm nylon mesh filter into a 50ml falcon tube. Using the plunger of a syringe, repeatedly mash the filter and rinse with cold Complete Medium up to 25ml.

Complete Media
DMEM + 10% FBS

10 Spin down 360xg for 10 min at 4°C. Acceleration 4, break 2.

11 Very carefully discard the supernatant.

12 Resuspend the cell pellet in 25ml of Complete Medium.

13 Spin down 360xg for 10 min at 4°C. Acceleration 4, break 2.

14 Very carefully discard the supernatant.

15 Add 1ml of 1x Red Blood Cell Lysis solution to your cell pellet.

16 Incubate 5 min on ice with periodic agitation.

17 Add fresh cold PBS up to 10ml.

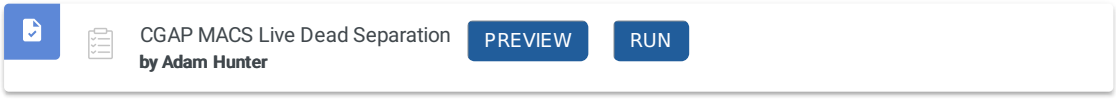
18 Pass through a 70µm cell strainer to remove any clumps still present.

19 Spin down 360xg for 5 min at 4°C.

20 Discard supernatant.

21 Resuspend pellet in 1ml of 0.04% BSA and count cells.

22 Proceed to “MACS Live Dead Separation”.



22.1

Material	Quantity	Supplier Info
15ml Falcon Tubes	3	Falcon (352097)
50ml Falcon Tubes	1	Falcon (352098)
MACS Dead Cell Removal Kit	1	Miltenyi Biotech (130-090-101)
Nuclease Free Water	19ml	Ambion (AM9939)
LS Columns	1	Miltenyi Biotech (130-042-401)
0.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.035)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)
PBS	10ml	GIBCO (14190-144)
Bovine Serum Albumin (BSA)	400ul	Sigma-Aldrich Co. Ltd (A7906-10G)

22.2 A single-cell suspension should have been prepared previously and cells number and viability assessed using 1:1 trypan blue dilution.
- A viability percentage below 70-80% usually justifies using this Dead Cell Removal protocol.

22.3 Remove required number of cells and place in a 15ml Falcon Tube.
- Required number of cells/total cells = volume required (ml).

22.4 Prepare 20ml 1X Binding Buffer by adding 1ml 20X Binding Buffer Stock to 19ml Nuclease Free Water.

22.5 Centrifuge cell suspension for 5min at 300g.

22.6 Remove supernatant.

22.7 Resuspend cell pellet in 100ul Dead Cell Removal MicroBeads per 10^7 cells.

22.8 Mix well and incubate for 15mins at room temperature.

22.9 When 5min of incubation remains, place MS column (if <2x108 cells) or an LS column (if <2x109 cells) on QuadroMACS Magnetic Cell Separator and run 500µl (MS column) or 3ml (LS column) 1X Binding Buffer through the LS column, using a waste 15ml Falcon Tube to catch the effluent.

2.10 When incubation is finished, add 1ml (MS column) or 3ml (LS column) 1X Binding Buffer to cells.

2.11 Run cell suspension through LS column on QuadroMACS Magnetic Cell Separator, using a 15ml Falcon Tube to catch effluent as the the live cell fraction.

2.12 When cells have passed through, run 4 x 500µl (MS column) or 4 x 3ml (LS column) 1X Binding Buffer through LS column on QuadroMACS Magnetic Cell Separator using the same falcon tube to catch effluent as the the live cell fraction.

2.13 Centrifuge cells at 500g for 5 min at 4°C. Resuspend in 0.5-1ml PBS + 0.04% BSA.

2.14 Count cells and viability using nucleocounter.

2.15 Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.

23 Proceed to 10X Preparation for scRNA sequencing.

24 Ensure all unused tissue, equipment and tubes that have been in contact with primary tissue are placed into Virkon in sweetie jar for a minimum of 1 hour. After this time aspirate and disposing in relevant sharps or waste routes.