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CGAP Human Lung Dissociation

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¹CGAP

Human Cell Atlas Metho...



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Protocol status: In development

We are still developing and optimizing this protocol

Created: June 15, 2018

Last Modified: July 11, 2018

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Material	Quantity	Supplier Info
DMEM + 10% FBS		???
Collagenase (10mg/mL stock) dilute in nuclease free water		Sigma Aldrich (C5138-25MG)
DNase I (0.5mg/mL stock)		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)
70nm 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 Cut lung into 1cm x 1cm sections and ensure weight is ~0.2g.
- 3 Transfer the piece of tissue to a 10cm petri dish and add ~250µl Digestion Medium to cover it.

Note**Digestion Media**

- **DMEM + Collagenase D (0.1ml/ml) + DNase I (10µl/ml)**

- 4 Using two scalpels, chop the piece inside the tube as finely as possible.
- 5 Add ~2ml of Digestion Media and transfer the mashed tissue to a 15ml falcon tube using a 5ml stripette.



- 6 Wash the dish with 1ml of Digestion Medium, transferring it to the 15ml falcon tube with the tissue.
- 7 Transfer it to an incubator at 37°C for 1 hour on a rocker (tissue from lower left lobe should be left for 30 minutes).

Note

Freeze the tissue samples at this stage

Protocol



NAME

CGAP Freezing Human Tissue in Isopentane

CREATED BY

Adam Hunter

PREVIEW

7.1

Isopentane	20ml	ThermoFisher (10468030)
Forceps	2	ThermoFisher UK Ltd (15232290)
100mm Petri Dish	1	Corning (430591)
Scalpel	1	Swann-Morton Ltd (0507)
Labelled 15ml falcon tubes	3 (Per Tissue)	Falcon (352097)
Small plastic weighing boats	3 (Per Tissue)	Fisher Scientific (HEA1420AF)

7.2 Receive tissue and place on ice.

7.3 Place the container of isopentane on dry ice.

7.4 Place 1 pair of forceps with the end in the dry ice.

7.5 Add lumps of dry ice into the isopentane to achieve the correct temperature -70°C for oesophagus, spleen, lung, liver and other organs (-40°C for striated muscle), monitoring with the -100°C thermometer.



- 7.6 Place labelled 15ml falcon tubes on dry ice.
- 7.7 Using the room temperature forceps, place the first tissue sample to be frozen onto the petri dish.
- 7.8 Cut tissue into chunks for freezing (1 for bulk RNA, 1 for spatial transcriptomics, 1 for fresh dissociation if not taken already).
- 7.9 Place each piece of tissue for freezing into a separate weighing boat
- 7.10 Using the cold pair of forceps, firmly pick up one side of the weighing boat and carefully lower the tissue directly into the isopentane (taking care to ensure the tissue doesn't float out of the weighing boat).
- 7.11 Hold the sample submerged in the isopentane for approximately 10-20 seconds (depending on the size of the sample) until there are no more bubbles produced from the tissue.
- 7.12 Drain as much of the isopentane off the weighing boat as possible.
- 7.13 Using the cold forceps, place each piece of tissue into the correspondingly labelled 15ml falcon tubes and keep on dry ice.
- 7.14 Transfer the labelled 15ml falcon tubes into the -80°C freezer.
- 7.15 Take the Mr Frosty cryo-container out of the dry ice and leave the lid off in a fume cupboard and allow isopentane to evaporate.
- 8 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.

Note**Complete Media
DMEM + 10% FBS**

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

- 10 Very carefully discard the supernatant.
- 11 Resuspend the cell pellet in 25ml of Complete Medium.
- 12 Spin down 360xg for 10 min at 4°C. Acceleration 4, break 2.
- 13 Very carefully discard the supernatant.
- 14 Add 1ml of 1x Red Blood Cell Lysis solution to your cell pellet.
- 15 Incubate 5 min on ice with periodic agitation.
- 16 Add fresh cold PBS up to 10ml.
- 17 Spin down 360xg for 5 min at 4°C.
- 18 Discard supernatant.
- 19 Resuspend pellet in 1ml of 0.04% BSA and count cells.
- 20 If percentage of live cells is higher than 70-80%, cells can then be processed for scRNA-seq.

If percentage of live cells is below 70-80%, remove dead cells by following "MACS Live Dead Separation".



Protocol



NAME

CGAP MACS Live Dead Separation

CREATED BY

Adam Hunter

PREVIEW

20.1

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)

20.2 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.

20.3 Remove required number of cells and place in a 15ml Falcon Tube.

- Required number of cells/total cells = volume required (ml).

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

20.5 Centrifuge cell suspension for 5min at 300g.

20.6 Remove supernatant.

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

- 20.8 Mix well and incubate for 15mins at room temperature.
- 20.9 When 5min of incubation remains, place MS column (if $<2 \times 10^8$ cells) or an LS column (if $<2 \times 10^9$ cells) on QuadroMACS Magnetic Cell Separator and run 500 μ l (MS column) or 3ml (LS column) 1X Binding Buffer through the LS column, using a waste 15ml Falcon Tube to catch the effluent.
- 20.10 When incubation is finished, add 1ml (MS column) or 3ml (LS column) 1X Binding Buffer to cells.
- 20.11 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.
- 20.12 When cells have passed through, run 4 \times 500 μ l (MS column) or 4 \times 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.
- 20.13 Centrifuge cells at 500g for 5 min at 4°C. Resuspend in 0.5-1ml PBS + 0.04% BSA.
- 20.14 Count cells and viability using nucleocounter.
- 20.15 Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.
- 21 Dilute cells to 2×10^6 cell per ml in 0.04% BSA and proceed to 10X Preparation for scRNA sequencing.
- 22 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.