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CGAP Human Lung Dissociation

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¹CGAP

Human Cell Atlas Method Development Community

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Protocol status: In development
We are still developing and optimizing this protocol

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13089

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	Material	Quantity	Supplier info
	DMEM + 10% FBS		???
	Collagenase (10mg/ml stock) dilute in nuclease free water		Sigma Aldrich (C5138-25MG)

	Scalpel	1	Swann-Morton Ltd (05507)
	100um Cell Strainers	2	ThermoFisher UK Ltd (153808001)
	50um Falcon Tubes	2	Falcon (3520998)

2.0 ml Syringe	1	ThermoFisher UK Ltd. (10673555)
70 nm Cell Strainer	1	ThermoFisher UK Ltd. (15370801)
15 ml Falcon Tubes	3	Falcon (352097)

Col PBS	Red Cell Lysis Buffer	0.5M DNase Free Water
50M	2M	1
GIBCO (14190-144)	Life Technologies Ltd. (00433357)	Epoch (0033008035)

Trypan Blue	20 μ l	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N001-50)

- 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 ~250 μ l Digestion Medium to cover it.

Note

Digestion Media

 - DMEM + Collagenase D (0.1ml/ml) + DNase I (10 μ l/ml)
- 4 Using two scalpels, chop the piece inside the tube as finely as possible.

- 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

	M a t e r i a l	Q u a n t i t y	S u p p l i e r i n f o
	15 m l F a l c o n t u i n e r s	3	F a l c o n t i n e r s (35520097)
	50 m l F a l c o n t u i n e r s	1	F a l c o n t i n e r s (35520098)
	M A C S D e a d C e l l R e m o v a l K i t	1	M i l t e n y i B i o t e c h (1330-0090-1001)

Nucleases Free Water	19M	Ambion (AM99339)
LS Columns	1	Milt Enyi Biotec (1300-442-401)
0.5M DNAL Bio Prep Buffer Tubes	1	Eppe ndorf (0033001008.0035)

	Trypan Blue	20ul	Fishers Scientific (11414815)
	C-Chips	1	Cambridge Bioscience (DHCC-NZ001-50)
	PBS	10ml	GIBCO (141190-144)

Bovine Serum Albumin (BSA)	400ul	Sigma-Aldrich Co. Ltd (A79906-10G)
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- 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 x 500 μ l (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.
- 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.