

# **CGAP Human Lung Dissociation**

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#### **Abstract**

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#### **Protocol**

## Step 1.

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 (00000011284932001)
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)

## Step 2.

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

## Step 3.

Transfer the piece of tissue to a 10cm petri dish and add 250µl Digestion Medium to cover it.



**Digestion Media** 

#### Step 4.

Using two scalpels, chop the piece inside the tube as finely as possible.

## Step 5.

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

#### Step 6.

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

## Step 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).



. CGAP Freezing Human Tissue in

## Isopentane

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## **₽** NOTES

Freeze the tissue samples at this stage

## Step 7.1.

Receive tissue and place on ice.

#### Step 7.2.

Place the container of isopentane on dry ice.

#### Step 7.3.

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

## Step 7.4.

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.

# Step 7.5.

Place labelled 15ml falcon tubes on dry ice.

## Step 7.6.

Using the room temperature forceps, place the first tissue sample to be frozen onto the petri dish.

## Step 7.7.

Cut tissue into chunks for freezing (1 for bulk RNA, 1 for spatial transcriptomics, 1 for fresh dissociation if not taken already).

## Step 7.8.

Place each piece of tissue for freezing into a separate weighing boat

#### Step 7.9.

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).

#### Step 7.10.

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.

#### Step 7.11.

Drain as much of the isopentane off the weighing boat as possible.

## Step 7.12.

Using the cold forceps, place each piece of tissue into the correspondingly labelled 15ml falcon tubes and keep on dry ice.

## Step 7.13.

Transfer the labelled 15ml falcon tubes into the -80°C freezer.

#### Step 7.14.

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.

## Step 7.15.

20ml	ThermoFisher (10468030)
2	ThermoFisher UK Ltd (15232290)
1	Corning (430591)
1	Swann-Morton Ltd (0507)
3 (Per Tissue)	Falcon (352097)
3 (Per Tissue)	Fisher Scientific (HEA1420AF)
	2  1  1  3 (Per Tissue)

## Step 8.

Collect the sample and filter the cells through a  $100\mu m$  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.

## NOTES

Complete Media DMEM + 10% FBS

## Step 9.

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

## Step 10.

Very carefully discard the supernatant.

## Step 11.

Resuspend the cell pellet in 25ml of Complete Medium.

## Step 12.

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

## Step 13.

Very carefully discard the supernatant.

## Step 14.

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

#### Step 15.

Incubate 5 min on ice with periodic agitation.

## Step 16.

Add fresh cold PBS up to 10ml.

#### Step 17.

Spin down 360xg for 5 min at 4°C.

## Step 18.

Discard supernatant.

## Step 19.

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

## Step 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

## . CGAP MACS Live Dead Separation

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## Step 20.1.

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.

## Step 20.2.

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

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

# Step 20.3.

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

## Step 20.4.

Centrifuge cell suspension for 5min at 300g.

## Step 20.5.

Remove supernatant.

## Step 20.6.

Resuspend cell pellet in 100ul Dead Cell Removal MicroBeads per 10<sup>7</sup> cells.

## Step 20.7.

Mix well and incubate for 15mins at room temperature.

#### Step 20.8.

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 500 $\mu$ l (MS column) or 3ml (LS column) 1X Binding Buffer through the LS column, using a waste 15ml Falcon Tube to catch the effluent.

## Step 20.9.

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

## Step 20.10.

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.

## Step 20.11.

When cells have passed through, run  $4 \times 500 \mu l$  (MS column) or  $4 \times 3 m l$  (LS column) 1X Binding Buffer through LS column on QuadroMACS Magnetic Cell Separator using the same falcon tube to catch effluent as the live cell fraction.

## Step 20.12.

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

## Step 20.13.

Count cells and viability using nucleocounter.

## Step 20.14.

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

## Step 20.15.

Ouantity	Supplier Info
3	Falcon (352097)
1	Falcon (352098)
1	Miltenyi Biotech (130-090-101)
19ml	Ambion (AM9939)
1	Miltenyi Biotech (130-042-401)
1	Eppendorf (0030 108.035)
20ul	Fisher Scientific (11414815)
1	Cambridge Bioscience (DHC-N01-50)
10ml	GIBCO (14190-144)
400ul	Sigma-Aldrich Co. Ltd (A7906-10G)
	1 1 19ml 1 1 20ul 1

#### Step 21.

Dilute cells to 2x10<sup>6</sup> cell per ml in 0.04% BSA and proceed to 10X Preparation for scRNA sequencing.

# Step 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.