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## CGAP Human Oesophagus Epithelium Dissociation

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Human Cell Atlas Metho...



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**Protocol status:** In development

We are still developing and optimizing this protocol

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**Keywords:** cgap human oesophagus epithelium dissociation, cgap, dissociation

## Troubleshooting

1

Material	Quantity	Supplier Info
100mm Petri Dish	1	Corning (430591)
Cold PBS	50ml	GIBCO (14190-144)
Forceps	2	ThermoFisher UK Ltd (15232290)
Scalpel	1	Swann-Morton Ltd (0507)
50ml Falcon Tubes	2	Falcon (352098)
15ml Falcon Tubes	1	Falcon (352097)
Dissociation Agent (Trypsin-EDTA 0.25%)	40ml	GIBCO (25200-056)
DNase I (Stock solution 10mg/ml)	400ul	Sigma (11284932001)
RPMI + 20% FBS	25ml	Gibco (42401042) + Sigma (F7524-50ML)
70um Cell Strainer	2	ThermoFisher UK Ltd (15370801)
2.0ml Syringe	2	ThermoFisher UK Ltd. (10673555)
1.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.051)
BSA	8µl BSA/ml PBS	Sigma Aldrich (A7906-10G)
0.5ml DNA LoBind Eppendorf Tubes	2	Eppendorf (0030 108.035)
Red Cell Lysis Buffer	5ml	Life Technologies Ltd. (00-4333-57)
Trypan Blue	40ul	Fisher Scientific (11414815)
C-Chips	2	Cambridge Bioscience (DHC-N01-50)

- 2 Receive oesophagus sample in solution.
- 3 Wash the samples with 10ml cold PBS to remove any residual contamination, stomach content and loose mucus.
- 4 Pour oesophagus onto 100mm glass petri dish and add another 10ml fresh cold PBS.



- 5 Open the samples longitudinally.
  - Epithelium should be a relatively loose, yellowish layer on the lumen side.
- 6 Using two forceps separate the epithelium from stroma. Place them onto separate 100mm petri dishes each with 10ml PBS.
  - From this stage on only process the epithelium. To process the mucosa/submucosa see protocol "".
- 7 Aspirate PBS.
- 8 In a few drops of PBS (~200µl), finely mince the epithelium using two scalpels simultaneously.
  - Too much PBS in the dish will make it more difficult to mince.
- 9 Add 4ml Trypsin-EDTA 0.25% to the dish and transfer the tissue to a 50ml falcon tube.
- 10 Wash the scalpel and the dish with a further 1ml of Trypsin-EDTA 0.25% and transfer it into the Falcon with the tissue.
- 11 Incubate the biopsies for 30 min at 37°C on a rocker.
- 12 Add 50µl DNase I dropwise to falcon to a final concentration of 100µg/ml.
- 13 Incubate at room temperature for 5 min.
- 14 Add 20ml of RPMI + 20% FBS to inactivate trypsin.
- 15 Centrifuge at 200g for 2 minutes.
- 16 Pass the cells through 70µm cell strainer into 50ml falcon tube.
- 17 Wash the Falcon Tube and the strainer with 5ml of RPMI with 20% FBS for total volume of 30ml (5ml of trypsin + 25ml of media).



- 18 Centrifuge at 500g for 5 minutes at 4°C. Remove supernatant.
- 19 Wash the cells with 5ml Cold PBS.
- 20 Centrifuge at 500g for 5 minutes at 4°C.
- 21 Add 1ml Red Cell Lysis buffer to the pellet and resuspend by racking/tapping.
- 22 Place on ice 5 min with periodic agitation.
- 23 Add 10ml of Cold PBS.
- 24 Centrifuge at 500g for 5 minutes. Remove supernatant
- 25 Resuspend in 1ml cold PBS with 0.04% BSA (8µl BSA/ml PBS).
- 26 Filter through a 70µm cell strainer.
- 27 Count cells and viability using nucleocounter.
- 28 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 "Dead cell removal EasySep kit".

## Protocol

NAME

**CGAP Dead cell removal EasySep kit with The Big Easy Magnet**

CREATED BY

Adam Hunter

Preview

28.1

Material	Quantity	Supplier Info
PBS	30ml	GIBCO (14190-144)
FBS	160ul	Sigma (F7524-50ML)
CaCl <sub>2</sub> (1mM)	1ul	VWR International Ltd (E506-100ML)
15ml Falcon Tubes	3	Falcon (352097)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)
0.5ml Eppendorf	1	Eppendorf (0030 108.035)
EasySep Dead Cell Removal Kit	1	StemCell Technologies (17899)
EasySep "The Big Easy" (grey) magnet	1	StemCell Technologies (18001)
Bovine Serum Albumin (BSA)	1ml	Sigma-Aldrich Co. Ltd (A7906-10G)

28.2 A single-cell suspension should have been prepared previously (e.g. by enzymatic dissociation of a tissue) and cells number and viability assessed using 1:1 trypan blue dilution. a. A viability percentage below 70-80% usually justifies using this Dead Cell Removal protocol.

28.3 Prepare 8ml of Recommended medium (PBS (8ml) + 2% FBS (160ul) + 1mM CaCl<sub>2</sub> (1ul)).

28.4 Centrifuge samples at 500g for 5 minutes.



- 28.5 Remove supernatant and resuspend in the appropriate volume of recommended medium (0.25 - 8ml) to obtain a suspension with  $1 \times 10^8$  cells/ml.a. If total number of cells is below  $2.5 \times 10^7$ , resuspend in the minimum volume, i.e. 0.25ml.
- 28.6 Transfer cell suspension to a 15ml Falcon.
- 28.7 Add Dead Cell Removal (Annexin V) Cocktail to sample:a. 50uL per ml of sample.
- 28.8 Add Biotin Selection Cocktail to sample:a. 50uL per ml of sample.
- 28.9 Mix (up and down with pipette) and incubate for 3 min at RT.
- 28.10 Vortex RapidSpheres™ for 30 seconds.a. Particles should appear evenly dispersed.
- 28.11 Add RapidSpheres™ to sample and mix:a. 100μL per ml of sample.b. No incubation, IMMEDIATELY move to next step.
- 28.12 Add Recommended medium to top up the sample to the indicated volume:a. Top up to 5ml for samples  $\leq 2$ ml.b. Top up to 10ml for samples  $> 2$ ml.
- 28.13 Mix by gently pipetting up and down 2 -3 times.
- 28.14 Place the tube (without lid) into the magnet and incubate for 3 mins at RT.
- 28.15 Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.a. Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.
- 28.16 Count cells and viability using 1:1 trypan blue dilution.
- 28.17 Add 5ml PBS with 0.04% BSA (200ul) to wash cells.



- 28.18 Centrifuge at 500g for 5 minutes.
- 28.19 Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.
- 29 Dilute cells to  $2 \times 10^6$  cell per ml in 0.04% BSA and proceed to 10X Preparation for scRNA sequencing.
- 30 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.