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CGAP Human Oesophagus Epithelium Dissociation - Tissue Stability

Forked from [CGAP Human Oesophagus Epithelium Dissociation](#)

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Human Cell Atlas Method Development Community

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Protocol status: Working
 We use this protocol and it's working

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Material	Quantity	Supplier Info
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100mm Petri Dish	1	Corning (430591)
Cold PBS	50ml	GIBCO (14190-144)
Forceps	2	ThermoFisher UK Ltd (15232290)
Scalpels	2	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 (1537 0801)
2.0ml Syringe	1	ThermoFisher UK Ltd. (1067 3555)
1.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.0 51)
BSA	267µl 7.5% BSA in 50ml PBS-/-	Sigma (A841 2)
0.5ml DNA LoBind Eppendorf Tubes	2	Eppendorf (0030 108.0 35)
Red Cell Lysis Buffer	5ml	Life Technologies Ltd. (00-4333-57)
Trypan Blue	40ul	Fisher Scientific (1141 4815)
C-Chips	2	Cambridge Bioscience (DHC-N01-50)

2 Receive oesophagus sample from mid-region in hypothermasol FRS solution (Sigma H4416).

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/mucosa should be a relatively loose, yellowish layer on the lumen side.
- 6 Using two forceps (scissors if necessary) separate the mucosa/epithelium layer from the stroma. Place them onto separate 100mm petri dishes each with 10ml PBS (this is a relatively crude separation protocol and is not 'pure' but avoids long overnight incubations in dispase).
 - From this stage on only process the mucosa/epithelium part for dissociation.
- 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 19ml Trypsin-EDTA 0.25% containing 100µg/ml DNase I to the dish and transfer the tissue to a 50ml falcon tube. (DNase is critical for this protocol). Note later tests indicated that this volume can be reduced to 5ml total.
- 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 Centrifuge sample 500g, 5 min at 4°C.
- 13 Add 20ml fresh 0.25% Trypsin-EDTA with 100µg/ml DNase and incubate at 37°C on rocker for a further 15min. (Note it was later determined that this second incubation step is not necessary for oesophagus, only for stomach, which causes a change in pH of the dissociation agent).
- 14 Pass through a 70µM cell strainer into a 50ml falcon tube.
- 15 Add 20ml of RPMI + 20% FBS to inactivate trypsin, mash the remaining tissue in the strainer with a syringe plunger and wash again with 5ml RPMI + 20% FBS. There is usually some undigested material remaining).
- 16 Centrifuge at 500g for 5 minutes, 4°C.
- 17 Wash cells with 5ml cold PBS-/-.
- 18 Centrifuge at 500g for 5 minutes, 4°C.
- 19 Add 5ml Red Cell Lysis buffer to the pellet and resuspend.

- 20** Place on ice 5 min with periodic agitation.
- 21** Add 10ml of Cold PBS-/-.
- 22** Filter through a 70µm cell strainer. (Often, small white filaments of undissociated material are still visible at this stage which must be removed before 10x loading).
- 23** Centrifuge at 500g for 5 minutes, 4°C. Remove supernatant.
- 24** Resuspend in 300-1000µl cold PBS with 0.04% BSA.
- 25** Count cells and viability manually using C-chip / trypan blue.
- 26** Proceed to 10X Preparation for scRNA sequencing.
- 27** 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.