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CGAP Human Oesophagus Epithelium Dissociation

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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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13088

1

	Material	Quantity	Supplier Info
	1000MP Petri Dish	1	Corning (430591)
	Cold PBS	50 ml	GIBCO (14190-144)
	Forceps	2	ThermoFisher UK Ltd (152332290)

	Scalpel	1	Swann-Morton Ltd (05507)
	50ml Falcon tubes	2	Falcon (352098)
	15ml Falcon tubes	1	Falcon (352097)

Disso- ciation Agent (Tryp- sin- EDTA 0.25%)	400 µl
DNAse I (Stock solution 10 mg/ ml)	400 µl
	Si- gna- l (1128849320001)
	GB- 2000- 5556)

RPM 1+200% FBS	25M	Gibco (42401042) + Sigma (F7552450ML)
70µm Cell Strainer	2	ThermoFisher UK Ltd (15370801)
2.0µm Syringe	2	ThermoFisher UK Ltd (10673555)

1.5 ml DNA LoBind Eppendorf Tubes	
1	8 µl BSA / ml PBS
Eppendorf (00300108051)	Sigma Aldrich (A7906-10G)

0.5 ml DNA Binding Buffer or Tubes	2	Appendix (003001008.035)
Red Cell Lysis Buffer	5 ml	Life Technologies Ltd. (00-43333-57)
Trypan Blue	40 ul	Fisher Scientific (11414815)

			C a m b r i d g e B i o s c i e n c e (D H C - N 0 1- 5 0)
	C - C h i p s	2	

- 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.

15 ml Falcon tubes	CaCl ₂ (1 mM)	FBS	PBS	GIBCO (14190-144)
3	1 ul	160 ul	300 ul	Sigma (F7524-50 ML)
Falcon (3520097)	VWR International (E506-100 ML)			

EasySep Dead Cell Removal Kit	1	Stem Cell Technologies (17899)
EasySep™ The Big Easy™ (grey) magnet	1	Stem Cell Technologies (18001)

Bovine Serum Albumin (BSA)	1m	Sigma-Aldrich Co. Ltd (A79006-10G)
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- 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 CaCl₂ (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 x 10⁸ cells/ml.a. If total number of cells is below 2.5 x 10⁷, 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. 100uL 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 $\leq$ 2ml.b. Top up to 10ml for samples > 2ml.
- 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 2×10^6 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.