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CGAP Human Oesophagus Epithelium Dissociation - Tissue Stability

Forked from CGAP Human Oesophagus Epithelium Dissociation

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Anna Wilbrey-Clark[1], Adam Hunter[2]

[1]Wellcome Trust Sanger Institute, HCA; [2]CGAP

Human Cell Atlas Metho...

Anna Wilbrey-Clark

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

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Troubleshooting

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	Material	Quantity	Supplier Info
	100 mm 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	400 ul	Sigma (112849

	solution 10mg/ml)		32001)
	RPMI + 20% FBS	25ml	Gibco (42401042) + Sigma (F7524-50ML)
	70um Cell Strainer	2	ThermoFisher UK Ltd (15370801)
	2.0 ml Syringe	1	ThermoFisher UK Ltd. (10673555)
	1.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.051)
	BSA	267 µl 7.5% BSA in 50ml PBS -/-	Sigma (A8412)

	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 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.