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CGAP Human Oesophagus Epithelium Dissociation

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[1]CGAP

Human Cell Atlas Method …

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Protocol status: In development
We are still developing and optimizing this protocol

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	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

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PREVIEW

28.1

	Material	Quantity	Supplier Info
	PBS	30ml	GIBCO (14190-144)
	FBS	160ul	Sigma (F7524-50ML)
	CaCl2 (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 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. 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 ≤ 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 $2x10^6$ 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.