Single-cell suspensions from primary human esophagus tissue

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ABSTRACT

A protocol to dissociate fresh esophagus tissue specimens for single-cell transcriptomics.

EXTERNAL LINK

https://www.southampton.ac.uk/medicine/about/staff/tju.page

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KEYWORDS

esophagus, esophageal, oesophageal, cancer, tissue, dissociation, single-cell, RNA, sequencing

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CREATED

Oct 03, 2018
MATERIALS TEXT

Equipment / reagents:
- Tubes - 50ml, 1.5ml (not non-stick), 0.5ml
- 40μm strainer: ThermoFisher (10737821)
- 70μm strainer: EASYstrainer (542070)
- Scalpel: Swann Morton No. 21 Sterile Disposable Scalpel (0507)
- Syringe plungers: Sigma (Z248010-1PAK)
- Centrifuge
- Incubator with shaking
- PBSA: Amphotericin B (ThermoFisher: 15290026) at a working concentration of 2.5 ug/ml in PBS
- 5ml DMEM complete:
  - 1 aliquot of Collagenase P (100ul at 150U/ml. Sigma: 112138857001) working concentration 3U/ml
  - 1 aliquot of DNAse (100ul at 2000U/ml. Sigma: 11284932001) working concentration 40U/ml
- DMEM (Sigma: D5671-500ml)
- 10% FBS (Pan Biotech: F40-37500)
- 1% LGlut (ThermoFisher: 25030081)
- 1% Penstrep (Sigma: P4333-100ml)
- DMEM empty
- RBC lysis solution:
  - 1ml RBC lysis reagent: ThermoFisher (00-4300-54)
  - 9ml H2O
- Trypsin-EDTA solution: Sigma (T3924-100ml)
- Trypan blue: Sigma (T8154-20ml)
- CChip: Labtech (DHC-F01)

SAFETY WARNINGS

Biological Hazard -
All biological samples should be treated as a possible cause of infection. Your departmental guidelines must be adhered to when handling human material. Working with primary cells may put the user at risk of exposure to blood-borne pathogens.

- Physical Hazard -
Scalpels are sharp. Care must be taken when using scalpels.

Always wear a lab coat and gloves

BEFORE STARTING
Work in a containment level 2 facility and safety cabinet

1. Wash tissue in PBSA.

2. Mince tissue with scalpel.
3. Transfer the minced tissue to the Collagenase/DNAse solution in a 50ml Falcon.

4. Shake the suspension at 110-150 rpm for 60 mins at 37°C (with the tube on its side to increase agitation of the tissue).

5. After 15, 30, and 60 mins – pipette up and down with descending sized pipettes (25, 10 and 5 ml).

6. Pipette thoroughly.

7. Strain with a 70 um cell strainer.

8. Add 10 ml DMEM (empty) to sieve and push through with syringe plunger.

9. Keep on ice from this point.

10. Spin 1500 rpm for 5 mins and remove media.

11. Resuspend pellet in RBC lysis buffer and incubate @ 4°C for 10 mins.

12. Add 10 ml DMEM (empty) and pipette up and down thoroughly.

13. Pass through 40 um cell strainer – pushing through with plunger.

14. Spin at 1500 rpm for 5 mins and remove media.

15. Resuspend pellet in 1 ml of cell suspension buffer.
16 In a 0.5ml tube, add 10 ul cell solution with 10 ul trypan blue.

17 Add resultant 20 ul mix onto disposable haemocytometer (C-Chip, FR type).

18 Count the cells and record cell viability