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Single-cell suspensions from primary human esophagus tissue

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CZI START Project

OPEN ACCESS

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ABSTRACT

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

GUIDELINES

Ensure all instruments needed are available and to hand before you start

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

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PROTOCOL integer ID:
16405

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MATERIALS

Equipment / reagents:

- Tubes - 50ml, 1.5ml (not non-stick), 0.5ml
- 40um strainer: ThermoFisher (10737821)
- 70um 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 concentraion 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 START INSTRUCTIONS

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 5mins and remove media.
- 11 Resuspend pellet in RBC lysis buffer and incubate @ 4°C for 10mins.
- 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