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Human Kidney / Tumour Tissue Disaggregation for Single Cell RNA Sequencing (10x Genomics platform)

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Kevin Loudon[1], John Ferdinand[1], Alexandra Riding[1], Menna Clatworthy[1]

[1]University of Cambridge

John Ferdinand
University of Cambridge

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

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Materials

STEP MATERIALS

Dead Cell Removal Kit **Miltenyi Biotec Catalog #**130-090-101

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Protocol materials

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Tissue Preparation

1 Take dissected tissue (renal cortex, medulla or tumour) and weigh tissue (typical biopsy size used 0.5 - 1 gram)

2 Pour approximately 2-3 mLs of "Digest Mix" onto sampe in 10cm^3 petridish

Note

PREPARATION OF DIGEST MIX

Ingredients
(1) RPMI alone
(2) Liberase TM (Sigma Aldrich)
(3) DNAse (Sigma Aldrich)

For 50mLs of RPMI add:
--> 625 microlitres of Liberase (Stock solution 2.5mg/mL)
--> 250 microlitres of DNAse (Stock solution 0.05mg/mL)

3 Using a razor blade mince into small pieces approximately 2mm^3 .

4 Transfer tissue into a gentleMACS C tube and add further 3-4 mLs of Digest mix.

Note

GentleMACS C tube by Miltenyi Biotec (Cat.130-096-334)

5 Place in shaking incubator at 37^{o}C for 30 minutes.

6 Homogenise sample in GentleMACS tube using program "Spleen 4" and "Lung 2" on GentleMACS dissociator.

7 Pass through a 100µm cell strainer with of a 2.5ml syringe plunger and wash through with cold running buffer.

Note

PREPARATION OF RUNNING BUFFER

Ingredients (for 1 litre)
(1) 1L PBS
(2) 5ml BSA (from reagent diluent kit)
(2) 4ml 0.5M EDTA

8 Centrifuge in a bench top centrifuge at 2000 RPM for 10 minutes and CAREFULLY remove the supernatant.

9 If sample is contaminated with red blood cells an additional red cell lysis step can be taken.

10 To ensure optimal yield for 10X Genomics single cell platform, a live cell enrichment step is required - this was performed using Miltenyi 'Dead Cell Removal Kit' (Please see manufacturers instructions for further details).

Note

LIVE CELL ENRICHMENT (Miltenyi - Dead Cell Removal Kit)

Ingredients
(1) Dead Cell removal Kit - Miltenyi (Order No. 130-090-101)
(2) MACS Column (LS or MS)

In brief for MACS colum LS
(1) Use LS column for 10^8 dead cells or 10^9 total cells.
(2) Remove supernatant completely following previous steps
(3) Resuspend pellet in 100 µL of *'Dead Cell Removal MicroBeads'* per approximately 10^7 total cells.
(4) Incubate 15 minutes at room temperature (20–25 °C).
(5) Rinse column with 1x binding buffer as per manufacturers instructions.
(6) Apply cell suspension in 1-10mLs of binding buffer and collect the effluent as the NEGATIVE cell population (i.e the live cells).
(7) Wash cells with PBS for 5 minutes at 1500rpm.

Dead Cell Removal Kit **Miltenyi Biotec Catalog #**130-090-101

11 Count the cells and resuspend the live cell supsension in appropriate volume of PBS for the 10X application.