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

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Abstract

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Protocol

Tissue Preparation

Step 1.

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

Tissue Preparation

Step 2.

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

NOTES

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)

Tissue Preparation

Step 3.

Using a razor blade mince into small pieces approximately 2mm3 .

Tissue Preparation

Step 4.

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

NOTES

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

Tissue Preparation

Step 5.

Place in shaking incubator at 37°C for 30 minutes.

Tissue Preparation

Step 6.

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

Tissue Preparation

Step 7.

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

NOTES

PREPARATION OF RUNNING BUFFER

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

Tissue Preparation

Step 8.

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

Tissue Preparation

Step 9.

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

Tissue Preparation

Step 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).

READAGENTS

Dead Cell Removal Kit 130-090-101
by Miltenyi Biotec

NOTES

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.

Tissue Preparation

Step 11.

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