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Gut_All Cells_Human V.1

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Protocol status: Working
I use this protocol for dissociation of non-immune and immune cells from human colon samples and it works well.

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Abstract

This protocol has been tested by me on human adult colon samples and given good results for scRNAseq. I suggest that you do an enrichment step afterwards, because the dissociation can be quite variable results in terms of the fraction of immune vs non-immune cells that are retreived.

Materials

MATERIALS

- D-PBS (Without Ca++ and Mg++) 500 mL **STEMCELL Technologies Inc.** **Catalog #**37350
- Liberase DH **Roche** **Catalog #**5401054001
- Hyaluronidase **Merck Millipore (EMD Millipore)** **Catalog #**385931-25KU
- HBSS Media **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**41122100-007
- DNAse II **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D8764-30KU

1 Receive samples. Take about 4mm^2 sample for OCT

2 Wash samples twice with HBSS medium. In petri dish, mince sample into 0.5cm pieces.

3 Incubate the samples in 2ml HBSS medium containing 1.07 Wunsch units/ml (2.14wu) Liberase DH (Roche Applied Science, Germany) and 70 U/ml hyaluronidase (Calbiochem/Merck, Germany) for 45 min at 37C on a shaking platform at 600 rpm.

4 Homogenise the sample every 15 min the solution by pipetting up and down with a 1ml pipette for 1 min.

5 Pass cells through a 40mM sieve. Spin them down at 300 g at 4C for 10 min.

6 Resuspended in 300ml PBS buffer containing 100 IU/ml DNAse II (Sigma Aldrich, Missouri, USA).

7 Proceed with bead enrichment as per manufacturers protocols. Typically this will be a Miltenyi CD45+ enrichment.

8 Count cells and dilute to the desired concentration with PBS.

9 Load cells (aim for 5000/run) on to the 10X.

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