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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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Gut_All Cells_Human V.1

Kylie James¹

¹Wellcome Sanger Institute

Kylie James
Wellcome Sanger Institute

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 retrieved.

MATERIALS

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⊗ D-PBS (Without Ca++ and Mg++) 500 mL **Stemcell Technologies Catalog #37350**

⊗ Liberase DH **Roche Catalog #5401054001**

⊗ Hyaluronidase **Merck Millipore Catalog #385931-25KU**

⊗ HBSS Media **Sigma Catalog #41122100-007**

⊗ DNase II **Sigma Aldrich Catalog #D8764-30KU**

- 1 Receive samples. Take about 4mm² 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 40µm 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.