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Protocol status: Working
 We use this protocol currently in our group for single cell analysis and a more thorough flow cytometric analysis of human liver non-parenchymal cells with our main emphasis being on mononuclear phagocytes (Kupffer cells, macrophages, monocytes and Dendritic cells).

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Protocol for the isolation of non-parenchymal liver cells from human liver biopsies (1-2cm3)

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Human Cell Atlas Method Development Community

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ABSTRACT

Optimised protocol for the isolation of non-parenchymal cells from human liver biopsies. Used on 1-2cm3 biopsies.

GUIDELINES

Process tissue ASAP after removal from patient to avoid excessive cell death.

MATERIALS

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✕ conical tubes, 50ml **Contributed by users**

✕ RPMI 1640 (with L-glutamine and sodium bicarbonate) **Sigma Aldrich Catalog #R8758**

✕ DNase I recombinant, RNase-free **Sigma Aldrich Catalog #000000004716728001**

✕ PBS **Contributed by users**

✕ Fetal bovine serum **Contributed by users**

✕ Corning® 100µm Cell Strainer **Corning Catalog #431752**

✕ Corning® 40µm Cell Strainer **Corning Catalog #431750**

✕ EDTA **Contributed by users**

✕ Collagenase A **Sigma Catalog #11088793001**

Dissociation

- 1 Put liver biopsy in a new 50ml tube
- 2 Cut finely with scissors
- 3 Add 20 mL RPMI containing enzymes (1mg/ml Collagenase A & 10U/ml DNase)
- 4 Put in shaking water bath at 37 °C for 00:20:00 5 – shake vigorously every 00:05:00 by hand
- 5 Add 20 mL cold PBS and place on ice
- 6 Filter through 100um filter
- 7 Spin down 400g 00:05:00

- 8 Remove Supernatant
- 9 Lyse RBCs if required (add 4ml RBC lysis buffer, incubate 4 °C for 00:03:00 , add 20 mL PBS and spin down as per step 7)
- 10 Resuspend cells in FACS buffer (2% FCS, 2mM EDTA, PBS) and count
- 11 Spin down as per step 7 and resuspend at desired concentration ($2-5 \times 10^6$ in 200ul for flow cytometry staining)
- 12 Filter through 40um filter and put on plate/in tube for staining
- 13 Spin down as per step 7 and proceed with staining for flow cytometry as required

FACS for Single Cell Analysis

- 14 Make antibody mix:
 1. For Live CD45+ enrichment:
 - Anti-Human CD45 APC-Cy7 Biolegend 368516 5ul/test (1 test = 5×10^6 cells in 100ul PBS)
 - Fixable Live/Dead Viability Dye Stain Thermo Fischer 65-0866-18 1:300 in PBS.
 2. For monocyte-macrophage enrichment:
 - Anti-Human CD45 APC-Cy7 Biolegend 368516 5ul/test (1 test = 5×10^6 cells in 100ul PBS)
 - Fixable Live/Dead Viability Dye Stain Thermo Fischer 65-0866-18 1:300 in PBS.
 - Anti-Human CD14 AF488 Biolegend 301804 5ul/test

- Anti-Human CD16 PE-Dazzle 594 Biolegend 302054 5ul/test

- 15 Stain sample 5×10^6 cells in 100ul PBS + Antibodies for 00:30:00 at 4 °C
- 16 Add 5ml FACS buffer to wash (if staining in a tube) or 100ul FACS buffer to wash (if staining in a plate)
- 17 Spin down as per step 7
- 18 Resuspend in 1-2ml FACS buffer and proceed to FACS to sort cells as Live CD45+, and CD14+CD16-, CD14+CD16+ and CD14-CD16+.