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

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Charlotte Scott[1], Martin Guilliams[1]

[1]VIB-UGent Center for Inflammation Research, Ghent, Belgium

Human Cell Atlas Metho...

Charlotte Scott

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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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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

MATERIALS

- conical tubes, 50ml
- RPMI 1640 (with L-glutamine and sodium bicarbonate) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R8758
- DNase I recombinant, RNase-free **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**000000004716728001
- PBS
- Fetal bovine serum
- Corning® 100µm Cell Strainer **Corning Catalog #**431752
- Corning® 40µm Cell Strainer **Corning Catalog #**431750
- EDTA
- Collagenase A **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**11088793001

Troubleshooting

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 (2-5×10^6 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+.