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.

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).

GUIDELINES

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

MATERIALS

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

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 (2-5x10^6 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 = 5x10^6 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 = 5x10^6 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 5x10^6 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+.