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

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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

- 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

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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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**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

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5. Add 20 mL cold PBS and place on ice.

6. Filter through 100um filter.

7. Spin down 400g for 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

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+.