APR 12, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgbzpz3gpk/v1

Collection Citation: Dorien De Pooter, Ben De Clerck, Koen Dockx, Domenica De Santis, Sarah Sauviller, Pascale Dehertogh, Matthias Beyens, Isabelle Bergiers, Isabel Nájera, Ellen Van Gulck, Nádia Conceição-Neto, Wim Pierson 2024. Robust isolation protocol for mouse leukocytes from blood and liver resident cells for immunology research. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbzpz3gpk/v1

Robust isolation protocol for mouse leukocytes from blood and liver resident cells for immunology research

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ABSTRACT

Research on liver-related conditions requires a robust and efficient method to purify viable hepatocytes, lymphocytes and all other liver resident cells, such as Kupffer or liver sinusoidal endothelial cells. Here we describe a novel purification method using liver enzymatic digestion, followed by a downstream optimized purification. Using this enzymatic digestion protocol, the resident liver cells as well as viable hepatocytes could be captured, compared to the classical mechanical liver disruption method. Moreover, single-cell RNA-sequencing demonstrated higher quality lymphocyte data in downstream analyses after the liver enzymatic digestion, allowing for studying of immunological responses or changes. In order to also understand the peripheral immune landscape, a protocol for lymphocyte purification from mouse systemic whole blood was optimized, allowing for efficient removal of red blood cells. The combination of microbeads and mRNA blockers allowed for a clean blood sample, enabling robust single-cell RNA-sequencing data. These two protocols for blood and liver provide important new methodologies for liver-related studies such as NASH, hepatitis virus infections or cancer research but also for immunology where high-quality cells are indispensable for further downstream assays.

Protocol status: Working
We use this collection and it's working

Created: Apr 01, 2024

Last Modified: Apr 12, 2024

COLLECTION integer ID: 98109

Keywords: Liver, Hepatocytes, NPC, IHL, HBV, Hepatitis, immunology, RNAseq, blood, GentleMACS

MATERIALS

Reagents:

- RPMI1640 medium with L-glutamine (Lonza, BE12-702F)
- FCS, frozen (0.22µm filtered Gibco by Thermo Fischer Scientific, 011-90005M)
- 1×
  Dulbecco's PBS (without calcium magnesium) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537
- 10×PBS DPBS **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1408
- Ethanol ≥70% (v/v) TechniSolv® **VWR International Catalog #**83801.360
- UltraPure DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977035
- DMEM(1×) + 4.5g/l D-glucose + L-glutamine
  DMEM, high glucose **Thermo Fisher Catalog #**41965039
- Percoll **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**17-0891-01
- William's E Medium, no phenol red **Thermo Fisher Catalog #**A1217601
- Debris Removal Solution **Miltenyi Biotec Catalog #**130-109-398
- Trypan Blue **Invitrogen - Thermo Fisher Catalog #**T10282
- Cellaca AOPI Viastain **Nexcelom Catalog #**CS2-0106-25mL
- UltrapPure 0.5M EDTA pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15575020
- 10% MACS BSA Stock Solution **Miltenyi Biotec Catalog #** 130-091-376
- TheraPEAK ACK Lysing Buffer **Lonza Catalog #**BP10-548E

Equipment:

- gentleMACS Octo Dissociator with Heaters **Miltenyi Biotec Catalog #** 130-096-427
- Surgical instrument set (scissors and tweezers)
- Sharps container 1.5L (BD, 305624)
- Pipettors and tips
- Pipette controller
- Serological pipettes
- Water bath (VWR, VWRI462-0057)
- Lab Armor™ Beads **Thermo Fisher Catalog #**A1254302

- VACUSAFE aspiration system, 4L PP bottle, tubing fittings (Integra Biosciences, 391-2094)
- Bottle 4L, PP with closed lid (Integra Biosciences, 158370)
- Centrifuge with swinging buckets
- Microscope
- Cellaca-MX-AOPI cell counter (Nexcelom)

Materials:

- BD Plastipak Luer-lok syringe 20 mL (BD, 300629)
- 23G needle HSW HENKE-JECT (Henke Sass Wolf, 4710006025)
- Liver Perfusion Kit, mouse and rat **Miltenyi Biotec Catalog #**130-128-030 containing:

1. 1 vial of Enzyme D (lyophilized powder)
2. 1 vial of Enzyme R (lyophilized powder)
3. 1 vial of Enzyme A (lyophilized powder)
4. 100 mL Buffer P (20×)
5. 1 mL Reagent C
6. 1 mL Reagent E

- gentleMAC Perfusers **Miltenyi Biotec Catalog #**130-128-151
- gentleMACS Perfusion Sleeves **Miltenyi Biotec Catalog #**130-128-752
- GentleMACS C Tube **Miltenyi Biotec Catalog #**130-096-334
- MACS SmartStrainers (100 µm) **Miltenyi Biotec Catalog #**130-098-463
- Tissue Culture Dish (diameter 60 mm) (Falcon, 353002)
- Tissue Culture Dish (diameter 100 mm) (Falcon, 353003)
- Falcon Round-Bottom polystyrene tubes 5 mL (Falcon, 352054)
- Falcon 15 mL Polypropylene conical Tube (Falcon, 352097)
- Falcon 50 mL Polypropylene conical Tube (Falcon, 352070)
- Counting slides (Glasstic Slide 10 with grids, Kova, 87144E)
- 96-well plate U-bottom
- Cell strainer 100 µm (Falcon, 352360)
- Celltrics 50 µm strainer (Sysmex, 04-004-2327)
- Cellaca counting plates (Nexcelom, CHM24-A100-004)

FILES

SEARCH

Protocol

NAME

Mechanical liver dissociation

VERSION 1

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OPEN →

Protocol

NAME

Enzymatic liver dissociation (with liver perfusion kit)

VERSION 1

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Ex vivo cell isolation

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Protocol

NAME

Blood sampling, cell isolation, single-cell GEM-generation, globin mRNA blockers and sequencing library preparation protocol

VERSION 1

CREATED BY

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