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Ex vivo cell isolation



In 1 collection

Dorien De Pooter¹, Ben De Clerck¹, Koen Dockx², Domenica De Santis², Sarah Sauviller¹, Pascale Dehertogh¹, Matthias Beyens³, Isabelle Bergiers³, Isabel Nájera⁴, Ellen Van Gulck¹, Nádia Conceição-Neto¹, Wim Pierson¹

¹ID Discovery, Infectious Diseases Therapeutic Area, Janssen Research and Development, Beerse, Belgium;

²Charles River Laboratories, Beerse, Belgium;

³Discovery Technologies & Molecular Pharmacology, Therapeutics Discovery, Janssen Research and Development, Beerse, Belgium;

⁴ID Discovery, Infectious Diseases Therapeutic Area, Janssen Research and Development, California, Brisbane, USA



Wim Pierson

ID Discovery, Infectious Diseases Therapeutic Area, Janssen ...

ABSTRACT

This protocol details ex-vivo cell isolation.

MATERIALS

Reagents:

- RPMI1640 medium with L-glutamine (Lonza, BE12-702F)
- FCS, frozen (0.22µm filtered Gibco by Thermo Fischer Scientific, 011-90005M)
- 10×PBS X DPBS Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1408
- Percoll Merck MilliporeSigma (Sigma-Aldrich) Catalog #17-0891-01
- - Dulbecco's PBS (without calcium magnesium) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D8537
- Trypan Blue Invitrogen Thermo Fisher Catalog #T10282
- 10% X MACS BSA Stock Solution Miltenyi Biotec Catalog # 130-091-376
- William's E Medium, no phenol red Thermo Fisher Catalog #A1217601
- TheraPEAK ACK Lysing Buffer Lonza Catalog #BP10-548E

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Reagent preparation:

- 1 PBS-2% FCS: Thaw an aliquot of filtered FCS and prepare a solution of 2% FCS (vol/vol) in 1x PBS.
- 2 33.75% Percoll gradient: Prepare an isotonic solution of 33.75% Percoll using 10x PBS and 1x PBS-2% FCS.

 Prepare this solution fresh and store at Room temperature and protect from light.
- 3 1x Debris removal solution: Prepare a 1:2 dilution of Debris removal solution in cold 1x PBS.
- 4 Trypan blue: Filter Trypan blue solution using a 100 μm cell strainer and dilute 1:2 in William's E medium.
- MACS buffer: MACS buffer is a solution containing PBS pH 7.2, 0.5% BSA and 2 mM EDTA. Prepare this by diluting 10% BSA solution 1:20 and 0.5M EDTA 1:250 with 1x PBS.
- 6 PBS-0.04% BSA: PBS-0.04% BSA solution is prepared by diluting 10% BSA solution 1:250 in 1x PBS.

7 Culture medium: Add 🚨 50 mL of FCS to 🚨 500 mL of RPMI1640 with L-Glutamine.

Procedure: PART I

15m

5m

8 Liver: Separation hepatocytes from non-parenchymal cells



8.1 Centrifugate the C-tubes containing liver dissociate at \$\epsilon\$ 50 x g, 4°C, 00:05:00 . Set the



acceleration at 9 and brake at 5.

After centrifugation, a big brown pellet should be visible which contains most hepatocytes. Take as much of the supernatant as possible without disturbing the hepatocytes (proceed to Section I.A). The supernatant contains the NPC; transfer it to a new 15 mL tube (proceed to Section I.B).

9 I.A) Hepatocyte purification

Note

CRITICAL! Always keep the hepatocytes | On ice | and work with cold buffers and solutions

- 9.1 Resuspend the hepatocyte pellet in 🚨 3 mL of William's E medium.
- 9.2 Underlay the cell suspension carefully with 4 6 mL of 1x debris removal solution by passively dispending it slowly with a pipette controller at the tube bottom to avoid mixing of the phases.

9.3

Centrifugate at \$\iint 500 \text{ x g, 4°C, 00:10:00}\$

10m



- 9.4
- Aspirate the supernatant completely and resuspend the cell pellet to single cells in 4 nmL cold William's E medium. After resuspension, add 🚨 4 mL | cold William's E medium.

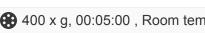
10 I.B) Non-Parenchymal Cells (NPC) purification

Note

IMPORTANT! For 10X sequencing, keep cells at \$\mathbb{

10.1

Centrifugate the 15 mL tube containing the NPC at 400 x g, 00:05:00 , Room temperature 5m



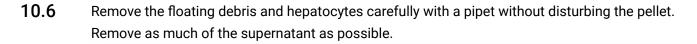


- 10.2 Discard supernatant by pouring, resuspend the NPC in 8 mL of 33.75% Percoll gradient.
- 10.3

brake at 3.

10.4

During centrifugation, prewet Celltrics 50 µm strainer with 4 500 µL PBS-2% FCS.



Note

The type of downstream assay will determine the following steps.

11 I.B.1) Flow Cytometry

- 11.1 Resuspend the NPC in Δ 1 mL PBS-2% FCS and transfer cells over 50 μm cell strainer in a new 5 mL polystyrene round bottom tube.
- 11.2 Rinse tube and filter with an additional 4 2 mL PBS-2% FCS.





- *****
- 11.4 Discard supernatant and resuspend in 4 1 mL PBS-2% FCS and count cells.

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12 I.B.2) For 10X sequencing:

12.1 Resuspend pellet in 🚨 1 mL of cold ACK buffer and incubate for 🕙 00:05:00 🕴 On ice



To stop the lysis, add Δ 2 mL of cold PBS and transfer cells over 50 μm cell strainer in a new 5 mL polystyrene round bottom tube.

12.3 Rinse the filter and tube with \triangle 500 µL cold PBS-2% FCS.



12.4 Centrifugate at **❸** 400 x g, 4°C, 00:05:00 .



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12.5 Discard supernatant and resuspend cells in the appropriate volume culture media for cell counting.