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Cold dispase digestion of murine lungs improves recovery and culture of airway epithelial cells V.2

PLOS One Peer-reviewed method

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Spotlight series



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ABSTRACT

Airway epithelial cells (AECs) play a key role in maintaining lung homeostasis, epithelium regeneration and the initiation of pulmonary immune responses. To isolate and study murine AECs investigators have classically used short and hot (1h 37°C) digestion protocols. Here, we present a workflow for efficient AECs isolation and culture, utilizing long and cold (20h 4°C) dispase II digestion of murine lungs. This protocol yields a greater number of viable AECs compared to an established 1h 37°C dispase II digestion. Using a combination of flow cytometry and immunofluorescent microscopy, we demonstrate that compared to the established method, the cold digestion allows for recovery of a 3-fold higher number of CD45-CD31-EpCAM+ cells from murine lungs. Their viability is increased compared to established protocols, they can be isolated in larger numbers by magnetic-activated cell sorting (MACS), and they result in greater numbers of distal airway stem cell (DASC) KRT5+p63+ colonies in vitro. Our findings demonstrate that temperature and duration of murine lung enzymatic digestion have a considerable impact on AEC yield, viability, and ability to form colonies in vitro. We believe this workflow will be helpful for studying lung AECs and their role in the biology of lung.

The last step in this version contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.



MANUSCRIPT CITATION:

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

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MATERIALS

- Dissection scissors
- Blunt curved dissection tweezers
- · Spring bow scissors
- Elastic string
- Blunt G20 1" needle
- Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (DMEM/F12) (Gibco, 11320033)
- Penicillin-Streptomycin (Gibco, 15140122)
- Dulbecco's phosphate-buffered saline (DPBS) (Gibco, 14190144)
- 24-well plate (Costar, 3524)
- Collagen (Sigma-Aldrich, C8919-20ml)
- Fibronectin (Merck, F2006-1MG)
- Bovine Serum Albumin (BSA) (Sigma-Aldrich, A7030-500G)
- Hanks' Balanced Salt Solution (HBSS) (Gibco, 14025092)
- 70µm strainers (Miltenyi, 130-098-458)
- 40µm strainers (Miltenyi, 130-098-462)
- Dispase II (Sigma-Aldrich, D4693-1G)
- DNase I (Roche, 10104159001)
- 5ml Bijous
- 50ml falcon tubes
- ACK Lysing Buffer (Gibco, A1049201)
- Anti-CD45 MACS beads (Miltenyi, 130-052-301)
- Anti-CD31 MACS beads (Miltenyi, 130-097-418)
- Anti-EpCAM MACS beads (Miltenyi, 130-105-958)
- LS MACS columns (Miltenyi, 130-042-401)
- MS MACS columns (Miltenyi, 130-042-201)
- Fc block CD16/CD32 (BioLegend, 101301)
- OctoMACS separator (Miltenyi, 130-042-109)
- QuadroMACS separator (Miltenyi, 130-091-051)
- EDTA (Gibco, 15575020)
- Airway Epithelial Cell Growth Medium (Promocell, C-21060)
- DMH-1 (StemCell, 73632)
- A 83-01 (StemCell, 72022)
- Y-27632 (StemCell, 72302)
- CHIR9901 (StemCell, 72052)
- Trypan Blue (Gibco, 15250061)
- Stericup filter unit (Milipore, S2GPT01RE)
- 5ml syringes
- Haemocytometer

Recipes

MACS buffer wash - DPBS (no Mg^{2+} and Ca^{2+}) + 0.5% BSA + 2mM EDTA + 1% Pen/Strep

Well-plate coating solution - 3% collagen, 1% fibronectin and 1% BSA in HBSS. Distribute at least 0.2ml per 1cm². Incubate for at least 4-8h at 37°C, then keep up to 5 weeks at 4C° covered in film.

Airway epithelial growth media -1μ M A 83-01, 0.2 μ M DMH-1, 5 μ M Y27632 ROCK, 0.5 μ M CHIR9901 and 1% v/v Penicillin/Streptomycin in supplemented Promocell Airway Epithelial Cell Growth Medium. Keep up to 7 days at 4°C and protected from light.

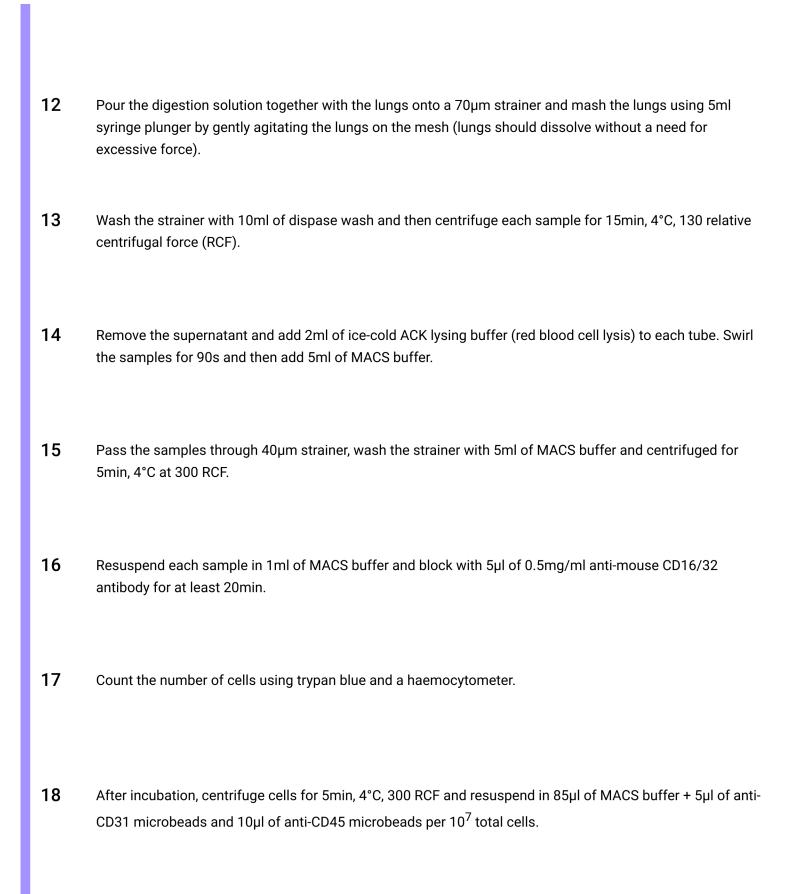
Digestion solution - 2mg/ml of Dispase II resuspended in sterile DMEM/F12 + 0.1mg/ml DNase I + 1% v/v Penicillin/Streptomycin filtered through 0.22μ m filter. Aliquot to Bijous (5ml) and freeze down. Thaw aliquots one hour before the start of the protocol.

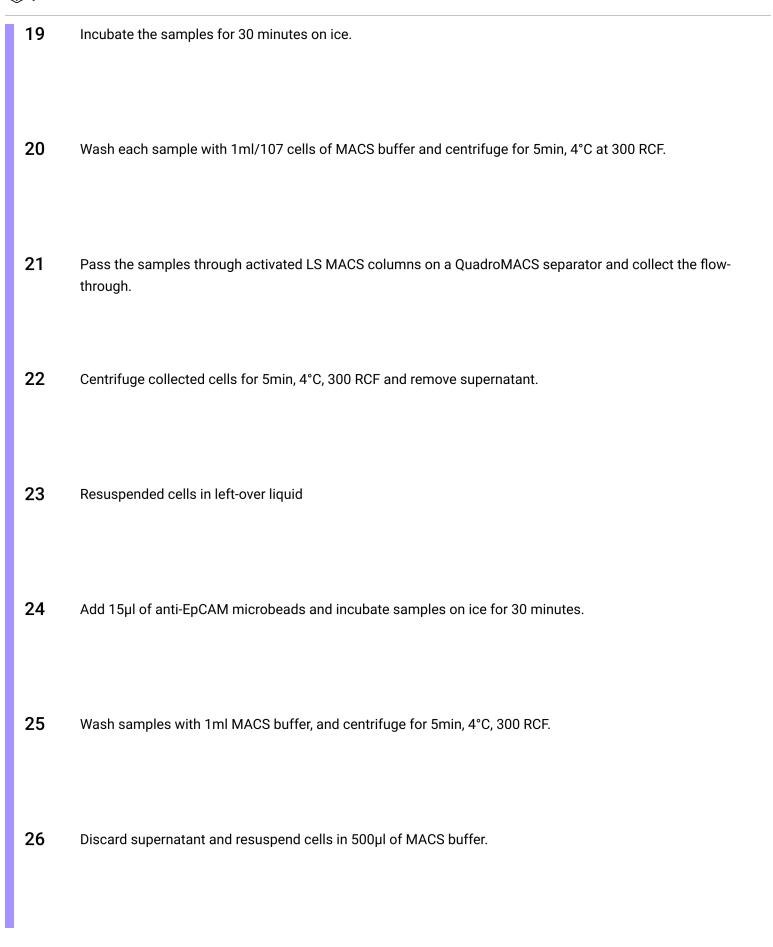
Dispase wash - 50µg/ml of DNasel with 1% v/v Penicillin/Streptomycin in DMEM/F12.

Cold dispase digestion of murine lungs improves recovery and culture of ...

- 1 Euthanise a mouse according to local protocols and regulations (excluding carbon dioxide).
- 2 Using scissors, cut the skin and open the abdomen. Follow through by cutting the skin along the sternum and neck.
- 3 Cut the abdominal aorta.

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27 Pass the cell suspension through activated MS MACS columns using an OctoMACS separator. 28 Flush out EpCAM+ cells into a fresh test tube from the column by applying 2ml of MACS buffer and inserting the plunger into the column. 29 Centrifuge cells for 5min at 4°C, 300 RCF, remove supernatant, and resuspend cell pellet in 5ml of supplemented airway epithelial growth media. 30 Count the number of live cells using trypan blue and a haemocytometer. 31 Coat the well plate with well-plate coating solution and incubate for at least 4-8h at 37°C before use. 32 Wash the coated 24-well plate with DPBS before adding 0.4ml of pre-warmed airway epithelial growth medium. 33 Add at least $2x10^5$ live MACS sorted cells per well and change media after 24h, followed by media changes every two days thereafter. Within 7 days p63+ KRT5+ colonies will appear. Spotlight video

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 $https://www.youtube.com/embed/A0m_ZunEEcs?si=bKH-5wWfZtM4xGsL$