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Isolation of ECs from Lymph node tissue for scRNAseq

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In Development

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ABSTRACT

Protocols describing the isolation of murine lymph node endothelial cells
Isolation of lymphatic endothelial cells for spectral flow cytometry and scRNA-seq
Basic analysis of spectral flow cytometry data of endothelial cells and scRNA-seq
Adapted from https://doi.org/10.1016/j.xpro.2022.101267

PROTOCOL CITATION

Guido Krähenbühl 2022. Isolation of ECs from Lymph node tissue for scRNAseq. **protocols.io** https://protocols.io/view/isolation-of-ecs-from-lymph-node-tissue-for-scrnas-b98wr9xe

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Preparation

1 Day before experiment check Antibodies to be used and prepare the following Reagents:

A	B	C
Dispase II	0.8 mg/mL	1 mL
Collagenase P	0.2 mg/mL	1 mL
DNase 1	0.1 mg/mL	1 ul
RPMI-1640		28 mL
Total		30 mL

Enzimatic Digestion solution for lymphnodes, store at 4C for 1 day (B is Final Concentration)

A	B	C
BSA	0.5%	2.5 g
EDTA, stock: 0.5M	2 mM	2 mL
PBS	-	498 mL
Total		500 mL

MACS buffer, store at 4C for up to 3 months (B is Final Concentration)

A	B	C
BSA	2%	10 mL
EDTA, stock: 0.5M	2 mM	2 mL
PBS	-	488 mL
Total		500 mL

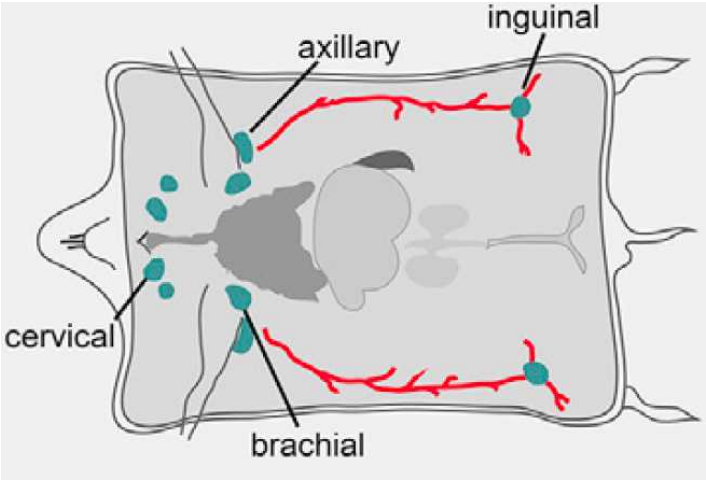
FACS buffer, store at 4C for up to 3 months

2 Before starting prepare:
Dissection Tools
If Brain tissue is also taken, prepare perfusion kit
5mL RPMI-1640
Keep all reagents on ice

Tissue collection 1h

3 Sacrifice mice using Isofluorine
(do not use cervical dislocation as this might affect perfusion of the brain)

4 Dissect inguinal, axillary, brachial and cervical lymphnodes



5 Perform transcardial perfusion via the left ventricle with **8 mL Ice-cold PBS** ice-cold at a perfusion rate of 2 mL/min for 5 min.

6 Dissect cervical lymph nodes and collect in **5 mL ice-cold RPMI-1640**

7 Collect remaining lymphnodes (axillary, brachial and inguinal) and add the to the cervical lymph nodes

8 **go to step #1 Repeat for a total of 5 mice to receive a sufficient number of cells (pool these samples)**

Tissue processing 35m

9 Remove the **RPMI-1640** and add **5 mL LN enzime solution** at **37 °C** **00:15:00** 15m

10 Aspirate and pipette the solution 10 times using a 5 mL serological pipette

11 Allow large fragments to settle down and transfer supernatant to 15m

20 mL ice-cold MACS buffer , add fresh **5 mL LN enzime solution** to remaining fragments and incubate at **37 °C** **00:15:00**
repeat 4 times or until fragments are fully digested
go to step #10

12 Centrifuge pooled supernatants at **300 x g, 4°C, 00:05:00** 5m

13 Resuspend in **5 mL MACS buffer**

14 Centrifuge at **300 x g, 4°C, 00:05:00**

15 Resuspent in **500 µL FACS buffer**

eryhtrocite lysis

16

Lyse erythrocytes with lysis buffer
(can result in loss of sensitive endothelial cells)

FACS Sort 30m

17 Incubation with blocking antibody for 10 min at 4°C (CD16/CD32, 1:100 in FACS buffer)

18 30m

Add Antibodies, mix and incubate at **4 °C** **00:30:00 in the dark**

CD31 Antibody	
CD45 Antibody	
Antibody 3	
Antibody 4	

19 Wash cells twice with FACS buffer by centrifugation at **300 rcf, 4°C, 00:05:00** and resuspend in **300 µL FACS Buffer** 5m

20 add viability dye immediately befor analysis (7-AAD at 1:100, DAPI at 1:100)

21 Gating and sorting