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Isolation of ECs from Brain tissue for scRNAseq on the 10x Chromium

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ABSTRACT

Protocols for endothelial cell isolation from mouse tissues: brain, addapted from

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Preparation

1 Day before experiment check Antibodies, Enzyme Mix 1 and 2 Reagents to be used and prepare the following Buffer:

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
20G needle and thin syringe
If Brain tissue is also taken, prepare perfusion kit
10mL DMEM Genta

A	B	C	D	E
	Enzime Mix 1		Enzime Mix 2	
	Buffer X	Enzyme P	Buffer Y	Enzyme A
1 Brain	1900 ul	50 ul	20 ul	10 ul
2 Brains	3800 ul	100 ul	40 ul	20 ul
5 Brains	9500 ul	250 ul	100 ul	50 ul

Keep all reagents on ice

Tissue Collection

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

4 Make an incision in the skin with fine scissors about a couple of millimeters above the orifice. From this opening the incision must continue on the mid ventral side up to the chin.

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

6 If cervical lymph nodes are needed disect them now, otherwise remove the Head and harvest Brain regions of interest and collect all Brains in **10 mL Ice-cold DMEM, genta**

7 If lymph nodes are also analysed **go to step #3** and repeat for up to 5 mice.
If only brain ECs are analysed a total of 2 mice should suffice

Tissue processing 15m

8 Mince Brain samples with a scalpel in a 10cm dish until they may pass through a cut 1000P pipette

9 Transfer minced sample to a 15 mL conical tube and add **1950 µL Enzime mix 1 per Brain** at **10 rpm, 37°C, 00:17:00** on the tube rotator

10 Add **30 µL enzime mix 2 per Brain**

11 Pipette 10 times up and down with a pasteur pipette (until the suspension passes without clogging)

12 Incubate at **10 rpm, 37°C, 00:12:00** on the tube rotator

13 Pass the cell supsension 10 times through a 20G needle on a 1mL syringe (dont create bubbles or foam)

14 Incubate at **10 rpm, 37°C, 00:10:00** on the tube rotator

15 Transfer cell suspension to a 50 mL conical tube, add 40 mL HBSS and filter through a 70 um cell strainer.

16 Centrifuge at **300 x g, 4°C, 00:05:00** 5m

16.1 Transfer supernatant to new 50 mL conical tube, keep pellet on ice and centrifuge supernatant at **300 x g, 4°C, 00:05:00** 5m

16.2 Transfer supernatant to new 50 mL conical tube, keep pellet on ice and centrifuge supernatant at **300 x g, 4°C, 00:05:00** 5m

17 Discard Supernatant and pool 3 pellets in **3600 µL MACS buffer** per Brain, add **400 µL Myelin Removal MicroBeads** per Brain and incubate at **4 °C** for **00:15:00** 15m

18 Add a total volume of **40 mL MACS buffer** and centrifuge at **300 x g, 4°C, 00:05:00** 5m
Prepare 6 times the number of LS columns per Brain by rinsing each with 3mL MACS buffer

19 Resuspend in **6 mL MACS buffer** per Brain sample and run 1mL per LS column (collect elluent)

19.1 Wash each column twice with 1mL MACS buffer (collect elluent)

20 Centrifuge total eluent at **300 x g, 4°C, 00:05:00** , remove supernatant 5m

21 Pool Pellets in **2 mL MACS buffer** per Brain and centrifuge at **300 x g, 4°C, 00:05:00** 5m

FACS sorting 15m

22 Resuspend the pellet in **500 µL FACS buffer** per brain

23 Prepare Controls and Samples with each antibody at 1/200:
Cells only
CD31
CD45
Viability dye
PDPN

24 Stain Cells for **00:30:00** at **4 °C** 30m

25 Add 3mL of FACS Buffer, centrifuge at **300 x g, 4°C, 00:05:00** and resuspend in 2 mL of FACS Buffer 5m