Isolation of microglia from adult mice (by Changsheng Wei, 1st draft, UNCORRECTED DRAFT)

According to protocol from PB lab, and doi:10.1016/j.xpro.2020.100035 and doi: 10.1038/nprot.2006.327

消化体系 Tissue dissociation enzyme With Neural Tissue Dissociation Kit (P) Cat. No. 130-092-628 (这个用于小鼠，Milyenyi / RWD 都有专用的成年鼠消化 KIT, 浙大高志华老师组建议不使用酶消化)
Enzyme mix 1 = 50ul Enzyme P + 1900ul Buffer X
Enzyme mix 2 = 10ul Enzyme A + 20ul Buffer Y

Dissociation using the gentleMACS™ Octo Dissociator with Heaters

1. Perfuse mice with cold HBSS and quickly isolate adult mouse brain in chilled solution. Determine the weight of tissue in 1 mL of HBSS (w/o).

2. Transfer 1950 µL of enzyme mix 1 for up to 400 mg of tissue into a gentleMACS C Tube (Miltenyi. Cat.No. 130-093-237).

3. Transfer mouse brain into the C Tube containing enzyme mix 1.

4. Transfer 30 µL enzyme mix 2 into the C tube.

5. Tightly close C tube and attach it upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters (Miltenyi. Cat.No. 130-095-937).
▲ Note: It has to be ensured that the sample material is located in the area of the rotator/stator.

6. Run the gentleMACS Program 37C_NTDK_1

7. After termination of the program, detach C Tube from the gentleMACS Dissociator. (此步骤后所有的操作尽可能在 4 °C)

8. Centrifuge briefly to collect the sample at the bottom of the tube. (300×g for 1 minutes at room temperature)

9. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (70 µm) placed on a 50 mL tube.
▲ Note: Moisten MACS SmartStrainer with buffer before use.
▲ Note: When upscaling the reagent volume and total volumes, increase also the number of MACS SmartStrainers (70 µm). One MACS SmartStrainer (70 µm) can be used for up to 2 mL.
▲ Note: Dissociated tissue can be removed from the closed C Tube by pipetting through

the septum-sealed opening in the center of the cap of the C Tube. Use ART 1000 REACH 1000 µL pipette tips.

▲ Note: Cells with a diameter >70 µm may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate mesh size.

在 50ml 管上使用 70um 过滤网。

（1）用过滤网过滤脑组织匀浆（约 2 毫升）。

（2）用 1XHBSS 冲洗消化管两次，第一次 23ml，第二次 2ml（共 45ml，仔细冲洗消化帽）

（3）转移 4ml 匀浆至提前装有 23ml 100% SIP (17-0891-09 GE Healthcare) 的 15ml EP 管中，混匀使整个匀浆为 30%Percoll (约 34%)。

stock isotonic percoll (SIP) 在室温下储存

100% SIP = 9 份 原 Percoll+1 份 10xHBSS

70% SIP = 7 份 100%Percoll+3 份 1xHBSS

Percoll Gradient Protocol

1. 用巴斯德管小心缓慢地将 2ml 70% SIP 注入至装有 30% SIP 匀浆的 15ml 管底部。在 70%-30%的交界处应能看到非常清晰的平面线。

Transfer 4 mL of the 37% SIP (from Step 15) to 15 mL conical tubes and slowly underlay 4 mL of 70% SIP (see Note 1). Then on top of the 37% layer slowly pipette 4 mL of 30% SIP, followed by 2 mL of HBSS. (PB lab Fudan)

2. Centrifuge gradient 40 min at 300×g (18 °C).

3. 使用移液管，从管顶部轻轻去除碎屑层，并用新的移液管将 5 2.0-3.0 ml 70%-30%的界面絮状物收集到一个干净的预装 108 ml 1X HBSS 的 15ml EP 管中。确保含有界面的 percoll 被**稀释约三倍**，通过倒置混合几次.

4. Centrifuge 7 min at 500×g at 4 °C

5. 弃上清，将沉淀重新悬浮于 1 ml HBSS+2%FBS 中，转移到 1.5 mlEP 管中，并 4°C 5000rcf 1 min 离心洗涤。

Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through a nylon mesh (MACS SmartStrainers (70 µm), # 130-098-462 or MACS SmartStrainers (30 µm), # 130-098-458) to remove cell clumps, which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2 – 8 ° C. Higher temperatures and/or

longer incubation times may lead to nonspecific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

3. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.

4. Add 10 µL of CD11b (Microglia) MicroBeads per 10^7 total cells.

5. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).

6. Wash cells by adding 1 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.

7. While samples are spinning, set up sorting columns in tissue culture hood
CRITICAL: Place the columns tightly in the magnet (must be fully in place).
Then place the ‘‘-’’ tubes that will be used to collect CD11b negative cells
under the columns. Top the columns with fresh nylon filters using autoclaved forceps to maintain sterility.

8. Prewash each column with 3 mL of MACS Buffer through nylon filter.
CRITICAL: Pre-wet each filter by adding 1 mL of HBSS (with calcium/magnesium) to the middle of the filter and then drag to side.

9. Remove samples from centrifuge and aspirate the supernatant. Resuspend pellets in 500 mL of MACS Buffer (HBSS+2%FBS)

10. Add cell suspension to nylon filter, and then rinse the old 15 mL tube with 2 mL of MACS Buffer and add it to the nylon filter.

11. Add 3 mL of MACS Buffer to column.

12. Once liquid has finished flowing through the columns, remove the ‘‘-’’ tubes and place on ice.

13. Remove the columns from the magnet, and place on top of ‘‘+’’ tubes that will be used to collect CD11b positive cells

14. Using a serological pipet, add 5 mL of MACS Buffer to the top of the column, and then quickly plunge column as hard as possible using the supplied plunger. Repeat this (for a total of 10 mL volume) for each column. Then place ‘‘+’’ tubes on ice with the ‘‘-’’ tubes

15. Centrifuge: 10 min, 300 3 g, 4 °C

16. Aspirate the supernatant (make sure to remove all bubbles, especially those from the ‘‘+’’ tubes)

CRITICAL: At this point you will only see a robust pellet in the ‘‘-’’ tubes and will not likely see a pellet in the ‘‘+’’ 15 mL falcon tubes. Because you likely will not see the pellet by eye, be sure to only displace the tube once to one side or the other and leave in place while aspirating off the supernatant (see Troubleshooting). Leave a meniscus of liquid covering the bottom of the tube prior to washing the pellet in 1X PBS, to avoid cell loss.

Labeling and flow cytometry

1. CD11b-FITC (Biolegend 101206 0.5ul in 100ul for 106 cells), CD45-PE (Biolegend 103106 1ul in 100ul for 106 cells), 染色 30min 4 °C;
2. 加入 1ml HBSS+2%FBS 洗涤，4 °C 450g 5min 离心弃上清。加入 HBSS+2%FBS 300μl 重悬，等待上机。