Isolation of Brain-infiltrating Leukocytes

1. Euthanize the mice and collect the maximal volume of blood through a cardiac puncture.

2. With the mice fixed on the dissecting board, make an abdominal incision in the peritoneum and open the thoracic cavity to expose the heart. Perfuse the mouse via the left (mouse left) (for brain; for lungs would be the right) cardiac ventricle: clip the right atrium of the heart with scissors and insert a 26-gauge needle (orange) into the left (mouse left) ventricle to administer 20 ml of cold PBS with a 20 ml syringe.

3. Harvest the brain and the spinal cord (count at least 30min) and put them into a 6 wells plate with RPMI alone. Cut the tissues in small pieces with scissors and then homogenate the tissues using two slides in a final volume of 5ml per organ.

   Collagenase D {Roche Ref: 11 088 882 001 (2.5g – 0.233 U/mg)} --> Stock concentration 500mg/ml.
   DNAse I {(Roche Ref: 11 284 932 001 (100mg)} --> Stock concentration 10mg/ml.

   Prepare 5ml RPMI with Collagenase D (0.5mg/ml final d=1/1000) and DNAse I (0.01mg/ml final d=1/1000) per organ.

   Incubate 30min at 37°C. Pipette up and down with 1ml blue tips.
   Then add EDTA to 2mM final pipette up and down with 1ml blue tips for 5min at RT.

4. Filter the cell suspension of the organ in a 70 µm cell strainer. Wash once @ 1500 rpm (480g) for 8 minutes, 4ºC.

5. Carefully resuspend the pellet in a 33.3% Percoll solution (v/v) – add 1.7 ml Percoll 100% + 3.3 ml of RPMI (without serum) at RT! - and transfer it into a 15ml Falcon tube.

6. **Centrifuge @2000 rpm (800 *g*) with no brake for 30 minutes at RT.** Gently aspirate the supernatant as much as possible, leaving only the cell pellet. Wash the pellet 2 times (the Percoll can inhibit the ACK red blood cell lysis buffer).

7. Ressuspend the pellet in 1 ml ACK and incubate at RT for 5 minutes to lyse the red blood cells.

8. Add 13 ml of FACS buffer and centrifuge @1500 rpm (480g) for 8 minutes (with the high-brake setting) at 8ºC.

9. Ressuspend the cells in complete medium and count the viable ones.

Cells are ready for flow cytometry staining.