**macs protocol for mouse opcs**

1. Add 13.5uL of 50mM B-mercaptoethanol to 10mL of Buffer X from papain-based neural tissue dissociation kit.

2. Prepare MACS buffer: 0.5% BSA, 0.5mM EDTA, 5ug/ml Insulin, & 1g/l glucose in DPBS.

3. Prepare OPC-A Media

4. Prepare OPC Differentiation Media

5. Remove the brains of postnatal day 3-5 mouse pups and place them in a petri dish containing HBSS without Ca+ and Mg+. Remove the meninges and place the dissected cortices in a pre-weighed dish pre-chilled with HBSS without Ca+ and Mg+. Weigh the cortices.

6. Prepare 1950µL enzyme mix 1 for up to 400 mg tissue. Mix 50µL Enzyme P and 1900µL Buffer X with β-ME and vortex. Pre-heat the mixture at 37 °C for 10–15 minutes before use.

7. Fire-polish three glass Pasteur pipettes so that decreasing tip diameters are achieved. The smallest opening should not be smaller than 0.5 mm so that the cells are not forced through with too much pressure. The edges should be rounded.

8. Transfer the cortices to a 0.5ml drop of HBSS in a fresh 10cm petri dish and dice them thoroughly with a razor blade. Add 5-6mL of HBSS to the petri dish and pipette into a 15mL conical tube. Rinse the dish to collect any remaining tissue and add it to the tube. For mice older than P10, cut 2–4 mm off the end of the pipette tip to facilitate pipetting.

9. Centrifuge at 300×g for 2 minutes at room temperature and aspirate the supernatant carefully.

10. Add 1950µL of pre-heated enzyme mix 1 per 400 mg tissue to the pellet, carefully avoiding the formation of air bubbles.

11. Incubate in closed tubes for 15 minutes at 37 °C under slow, continuous rotation or in 37oC water bath every 5min.

12. Prepare 30µL enzyme mix 2 per 400mg tissue by adding 20µL of Buffer Y to 10µL of Enzyme A. Add to sample & invert. Triturate slowly approximately 15X using the widest fire-polished pipette and carefully avoiding air bubbles.

13. Incubate at 37 °C for 10 minutes inverting frequently.

14. Dissociate tissue mechanically using the other two fire-polished pipettes in decreasing diameter. Pipette slowly up and down 10 times with each pipette, or as long as tissue pieces are still observable. Be careful to avoid the formation of air bubbles.

15. Incubate at 37 °C for 10 minutes inverting frequently.

16. Pre-wet a 40µm cell strainer by pouring 5ml of DPBS into a 50ml conical tube and then aspirating the liquid.

17. Add 10ml of HBSS with Ca+ and Mg+ to the 15ml tube containing the tissue. Apply the suspension to the strainer, collecting in the 50mL tube. {One filter for 2mL of suspension}.

18. Discard cell strainer and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate, leaving 2-5ml of supernatant in the tube.

19. Add 10ml of HBSS with Ca+ and Mg+ to the 50ml tube and centrifuge again at 300×g for 10 minutes at room temperature. Aspirate the supernatant completely and resuspend the pellet in 5ml of MACS buffer.

20. Remove a small aliquot for cell counting. Transfer the cell suspension to a 15ml tube and centrifuge at 300×g for 10 minutes at room temperature.

21. Count the cells by Trypan Blue visualization: Add equal volumes of 0.4% trypan blue stain & cell suspension. Pipette 10ul into the hematocytometer. Trypan blue will stain dead cells, whereas live cells where appear colorless and bright under phase contrast. {Expected yield: ~107 cells/brain}

22. After removing the supernatant, resuspend the pellet in 80ul of MACS buffer for every 107 cells. Add 10ul of FcR Blocking Reagent per 107 cells. Mix well, do not vortex, and incubate for 10min at 4oC.

23. Add 10ul of anti-O4 microbeads per 107 cells and mix well. Do not vortex. Incubate for 15min at 4oC.

24. Was cells by adding 2mL of MACS buffer per 107 cells. Centrifuge at 300×g for 10 minutes at room temperature. Aspirate the media completely and resuspend cells in 500uL of MACS buffer per 107 cells.

25. Prepare a MACS column by running 500uL MACS buffer through the column. Add up to 107 cells to the column. Wash the column 3X with 500ul of MACS buffer. Collect all flow-through in a 15ml conical tube (neurons and glial). Centrifuge flow-through at 300×g for 10 minutes at room temperature if you wish to keep these cells.

26. Remove the column from the magnet and place a fresh 15ml tube underneath. Elute the cells by adding 1ml pre-equilibrated OPC-A media to the column.

27. Count the cells. Plate in a PDL/laminin coated tissue culture plate and incubate at 37oC in 5% CO2.