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Dopamine neuron enrichment using MACS

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We use this protocol and it's working

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Abstract

high yield DA neuron sorting via MACS but using surface marker strategy described recently (**Kim et al.,BioRxiv**).

Troubleshooting

Cell dissociation

1 Use Worthington Papain Dissociation System (LK003150) to dissociate at day 25 of Studer lab dopamine neuron protocol. Add 32 mls of EBSS (vial 1) to the albumin ovomucoid inhibitor mixture (vial 4) and allow the contents to dissolve while preparing the other components. Mix before using and equilibrate within 5% CO2 incubator. Reconstitute for the first use, then store and reuse.

2 Add 5 mls of EBSS (vial 1) to a papain vial (vial 2). Place vial 2 in a 37°C water bath for ten minutes or until the papain is completely dissolved and the solution appears clear. If solution appears alkaline (red or purple) equilibrate the solution with 95% O2:5%CO2.

3 Add 500 µls of EBSS to a DNase vial (vial 3). Mix gently -- DNase is sensitive to shear denaturation. Add 250µls of this solution to the vial containing the papain. This preparation contains a final concentration of approximately 20 units/ml papain and 0.005% DNase.

4 Place 1ml papain solution per 6-well and incubate at 37°C, 5% CO2 for 45 min to 90 min, periodically agitating the plate.

5 Gently triturate the mixture with 10ml pipette

6 Carefully remove the cloudy cell suspension, place in sterile screw capped tube and centrifuge at 300g for 5 minutes at room temperature.

7 Mix 2.7 mls EBSS (vial 1) with 300µls reconstituted albumin-ovomucoid inhibitor solution (vial 4) in a sterile tube. Add 150 µls of DNase solution (vial 3)

8 Discard supernatant and immediately resuspend cell pellet in DNase dilute albumin-inhibitor solution

9 Discard the supernatant and immediately resuspend the pelleted cells in MACS buffer (PBS without Ca2+/Mg2+, 5% BSA, 2mM EDTA) and count cells

CD49 negative selection

10 Add CD49e-Biotin (Miltenyi, 130-110-532) at 1in50 dilution and incubate for 5-10 minutes at (2–8 °C). Note: 1) Incubating on ice can require longer staining times, 2) Neuron yield is directly affected by the quality of the differentiation, it is advisable to perform quality

control on your cells at day 11 and day 16 to assess % of on-target cells, 3) Yield may also be improved by performing a titration of the antibody

11 Wash cells twice by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

12 Resuspend cell pellet in 80ul per 10^7 total cells.

13 Add 20 µL of Anti-Biotin MicroBeads (Miltenyi, 130-090-485) per 10^7 total cells and incubate for 15 minutes at (2–8 °C).

14 Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

15 Resuspend $1.25×10^8$ cells in 500 µL of buffer (for LD columns)

16 Place LD Column (Miltenyi, 130-042-901) in the magnetic field of a suitable MACS Separator.

17 Prepare column by rinsing with 2 mL of buffer

18 Apply cell suspension onto the column.

19 Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer.

20 Collect total effluent; this is the unlabeled cell fraction (CD49e negative), Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

21 You may wish to collect CD49e positive fraction (off-target or neural progenitor cells), to do so, add 2×1ml buffer and gently use the provided plunger with the LD columns in a separate tube

22 Centrifuge cells 300xg for 10 minutes

23 Wash with MACS buffer and centrifuge 300xg for 10 minutes

CD184 postivie selection

24 Resuspend cells in 90ul MACS buffer and add 10 µl of a CXCR4-microbead antibody (Miltenyi, 130-100-070) per 10^7 cells for 10 minutes at (2–8 °C).

25 Scale volumes accordingly

26 Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

27 Resuspend up to 10^8 cells in 500 µL of buffer (LS columns)

28 Place LS column (Miltenyi, 130-042-401) in the magnetic field of a suitable MACS Separator.

29 Prepare column by rinsing with 3 mL MACS buffer

30 Apply cell suspension onto the column. Collect flow-through containing unlabeled cells (CD184 negative).

31 Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from

32 Note: Perform washing steps by adding buffer aliquots only when the column reservoir is empty.

33 Remove column from the separator and place it on a suitable collection tube.

34 Pipette the 5ml MACS buffer and immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. To increase the purity of magnetically labeled cells, the eluted fraction can be enriched over a second LS Column.

35 Centrifuge cells 300xg for 10 minutes

36 Resuspend cells in 1ml Day 25 media and count

37 Plate 150,000 CD49e negative / CD184 positive cells per cm2 in 4ml Day 25 media (Studer lab dopamine neuron protocol)