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Protocol status: Working
 We use this protocol and it's working

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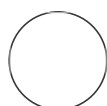
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Differentiation NPCs to Dopaminergic/Midbrain Neurons

In 1 collection

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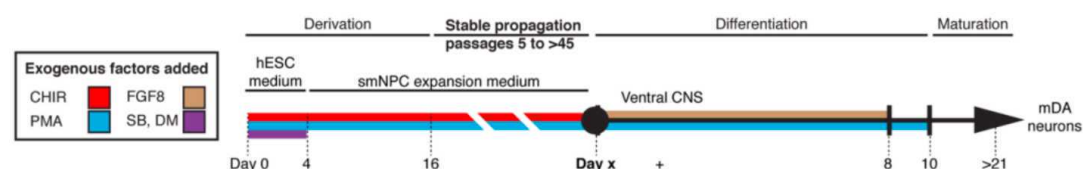


Federico Bertoli

ABSTRACT

This protocol details methods for differentiation of NPCs to Dopaminergic/Midbrain Neurons.

GUIDELINES



MATERIALS

Materials

Matrigel (Corning, #354230)
 DMEM/F12 without HEPES (Gibco #11320033)
 Accutase (Sigma Aldrich, #A6964-100 ml)
 Propagation/NPC-Medium
 Apol (Selleckchem, #S1049)
 Erhaltung/NPC-Medium
 Differentiation Medium
 Accumax (Gibco, #00-4666-56)
 Maturation Medium
 0.5µM PMA (Merck#540220-5MG)

Equipment

Centrifuge
 37°C Incubator
 Neubauer counting chamber

SAFETY WARNINGS



Please refer to Safety Data Sheets (SDS) for health and environmental hazards.


Day -2: split NPCs

35m

- 1 Coat numbers of wells you need on a well-plate with Matrigel:

Note

Matrigel (Corning, #354230)

Dilute  4 °C aliquot 1/10 in DMEM/F12 without HEPES (Gibco, #11320033)



-> there is always one aliquot thawed in  4 °C . More aliquots are in  -20 °C .

Keep Matrigel cool on ice!

- 1.1 Dilute thawed Matrigel out of  4 °C **1/10** in DMEM/F12 without HEPES.



Note



1ml diluted Matrigel is enough for one complete plate. Only rinsing the wells!

- 1.2 Incubate  00:30:00  37 °C in incubator.




30m





- 2 Remove old medium and add  500 µL Accutase (Sigma Aldrich, #A6964-100 ml) into one well of a 6well-plate or  250 µL Accutase for well of a 12 well-plate.

- 3 Incubate  00:05:00  37 °C in incubator.

5m


- 4 Dilute and inactivate Accutase with  1 mL DMEM/F12 without HEPES (Gibco #11320033) and collect in 15ml Falcon containing  3 mL DMEM/F12 without HEPES . Centrifuge  1100 rpm, 00:05:00 .

- 5 Remove supernatant and resuspend pellet in  1 mL Propagation/NPC-Medium + Apol (1/1000, Selleckchem, #S1049).

- 6 Count cells with Neubauer counting chamber (4big squares) and seed 1.000.000 cells in one matrigel-coated well of a 6well-plate. Use  1.5 mL Propagation/NPC-Medium + Apol (1/1000, Selleckchem, #S1049).
$$c/ml = \frac{\text{cells counted} \times 10 \times \text{dilution} \times 2}{(\text{dilution} \times \text{trypan blue}) \times 1000} \times 4$$

Day 0: Start Differentiation

- 7 Remove old Erhaltung/NPC-Medium and **add Differentiation-Medium**.

- 8 Change Medium every  48:00:00 .

2d


Day 6

5m

- 9 Coat numbers of wells you need on a well-plate with Matrigel:

Note

Matrigel (Corning, #354230)

Dilute  4 °C aliquot 1/10 in DMEM/F12 without HEPES (Gibco, #11320033)



-> there is always one aliquot thawed in  4 °C . More aliquots are in  -20 °C . **Keep**

Matrigel cool on ice!




9.1 Dilute thawed Matrigel out of  4 °C **1/10** in DMEM/F12 without HEPES.

Note

1ml diluted Matrigel is enough for one complete plate. Only rinsing the wells!

9.2 Incubate  00:30:00  37 °C in incubator.



10 Remove old medium and add  500 µL Accumax (Gibco, #00-4666-56) into one well of a 6well-plate. Incubate  00:05:00  37 °C in incubator.




5m

11 Dilute and inactivate Accumax with  1 mL DMEM/F12 without HEPES (Gibco #11320033) and collect in 15ml Falcon containing  3 mL DMEM/F12 without HEPES. Centrifuge  1100 rpm, 00:05:00.



12 Remove supernatant and resuspend pellet in  1 mL Differentiation-Medium + Apol (1/1000, Selleckchem, #S1049).

13 Count cells with Neubauer counting chamber (4big squares) and seed 1.000.000 cells in one matrigel-coated well of a 6well-plate. Use  1.5 mL Differentiation-Medium + Apol (1/1000, Selleckchem, #S1049).
$$c/ml = \frac{\text{cells counted} \times 10 \times \text{dilution} \times 2}{(\text{dilution} \times \text{trypan blue}) \times 1000} \times 4$$

Day 8: Start Maturation

14 Remove old Differentiation-Medium and **add Maturation-Medium + 0.5µM PMA** (1:2000, Merck #540220-5MG).

Day 10: Medium change

15 Change Medium *to Maturation (without PMA!)*.

16 Change Medium every  48:00:00 .

2d

Day 14: Final Splitting



5m

17 Coat numbers of wells you need on a well-plate with Matrigel:

Note

Matrigel (Corning, #354230)

Dilute  4 °C aliquot 1/10 in DMEM/F12 without HEPES (Gibco, #11320033)



-> there is always one aliquot thawed in  4 °C . More aliquots are in  -20 °C . **Keep**

Matrigel cool on ice!




17.1 Dilute thawed Matrigel out of  4 °C **1/10** in DMEM/F12 without HEPES.

Note

1ml diluted Matrigel is enough for one complete plate. Only rinsing the wells!



17.2 Incubate  00:30:00  37 °C in incubator.





18 Remove old medium and add  500 µL Accumax (Gibco, #00-4666-56) into one well of a 6well-plate. Incubate  00:05:00  37 °C in incubator.

5m



19 Dilute and inactivate Accumax with  1 mL DMEM/F12 without HEPES (Gibco #11320033) and collect in 15ml Falcon containing  3 mL DMEM/F12 without HEPES . Centrifuge  1100 rpm, 00:05:00 .

20 Remove supernatant and resuspend pellet in  1 mL Maturation-Medium + Apol (1/1000, Selleckchem, #S1049).

21 Count cells with Neubauer counting chamber (4big squares) and seed 1.000.000 cells in one matrigel-coated well of a 6well-plate. Use  1.5 mL Maturation-Medium + Apol (1/1000, Selleckchem, #S1049).
$$c/ml = \frac{\text{cells counted} \times 10 \times \text{dilution} \times 2}{(\text{dilution} \times \text{trypan blue}) \times 1000} \times 4$$

Day >21:

22 After Day 21, cells are ready.