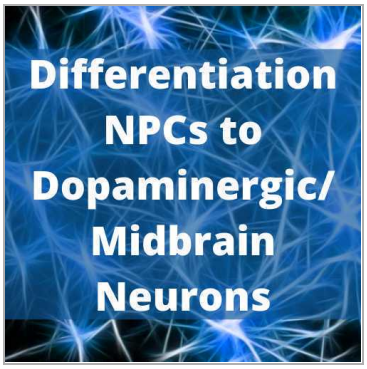

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Protocol status: Working
We use this protocol and it's working

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Differentiation NPCs to Dopaminergic/Midbrain Neurons

Forked from Differentiation NPCs to Dopaminergic/Midbrain Neurons

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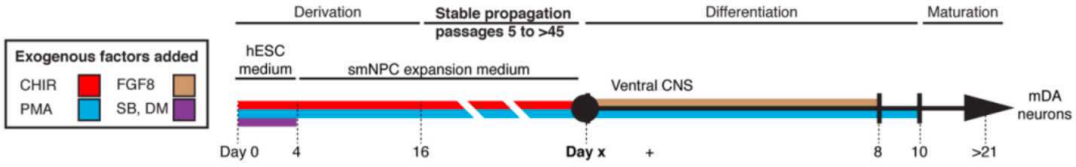
ABSTRACT

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{cells\ counted\ x\ 10\ x\ dilution\ x\ 2\ (dilution\ trypan\ blue)\ x\ 1000}{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{cells\ counted\ x\ 10\ x\ dilution\ x\ 2\ (dilution\ trypan\ blue)\ x\ 1000}{4}

Day 8: Start Maturation

14 Remove old Differentiation-Medium and ***add Maturation-Medium + 0.5uM 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{cells\ counted\ x\ 10\ x\ dilution\ x\ 2\ (dilution\ trypan\ blue)\ x\ 1000}{4}

Day >21:

22 After Day 21, cells are ready.