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Differentiation NPCs to Dopaminergic/Midbrain Neurons

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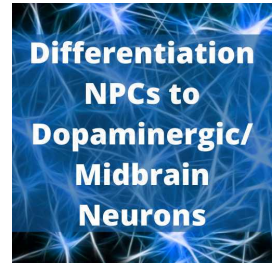
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Protocol status: Working

We use this protocol and it's working

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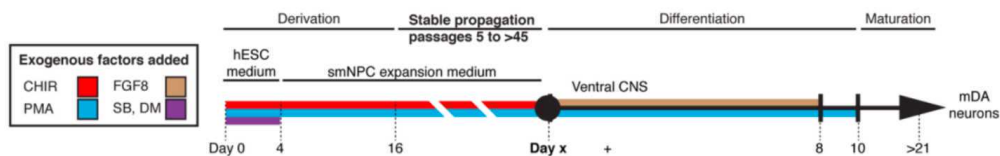
Protocol Integer ID: 79099

Keywords: differentiation, dxn, NPC, Dopaminergic, Midbrain, Neurons, ASAPCRN, differentiation of npc, midbrain neurons this protocol details method, midbrain neuron, dopaminergic, npc, differentiation,

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).

$$c/ml = \frac{cells\ counted \times 10 \times dilution \times 2\ (dilution\ trypan\ blue) \times 1000}{4}$$

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).

$$c/ml = \frac{cells\ counted \times 10 \times dilution \times 2\ (dilution\ trypan\ blue) \times 1000}{4}$$

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).

$$c/ml = \frac{cells\ counted \times 10 \times dilution \times 2\ (dilution\ trypan\ blue) \times 1000}{4}$$

Day >21:

22 After Day 21, cells are ready.