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Dopaminergic neuron differentiation V.1

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We use this protocol and it's working

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Abstract

This protocol has been used to differentiate dopaminergic neurons from hPSC adapted to feeder free culture systems.

Protocol overview

A. Plate preparation
B. Media recipes
C. Dopaminergic neuron differentiation

Attachments

PDF

Dopaminergic neuron ...

64KB

Materials

	Item	Vendor	Catalog number
	DMEM/F12	Gibco	11320033
	mTESR-plus Medium	StemCell Tech	100-0276
	Neurobasal medium	Gibco	21103049
	N2 supplement	Gibco	17502048
	B27 supplement without vitamin A	Gibco	12587-010
	L-Glutamine	Sigma	G8450
	Penicillin-Streptomycin	Gibco	15140122
	Y-27632 Dihydrochloride Rock inhibitor	ToCris	CD0141
	DPBS (No calcium, No magnesium)	Cytiva	SH30028
	Accutase	Innovative Cell Tech	AT104
	Matrigel	Corning	354230
	Laminin	R&D Systems	3400-010-02
	Poly-L-Ornithine	Sigma	P3655
	LDN	Stemgent	04-0074
	SHH C25II	R&D Systems	1845-SH-100
	SB431542	SelleckChem	S1067
	CHIR99021	ToCris	4423
	GDNF	PeProtech	450-10
	BDNF	PeProtech	450-02
	Dibutyryl-cAMP (Bucladesine)	SelleckChem	S7858
	Sodium L-Ascorbate	Sigma	A40-34
	TGFβ3	R&D Systems	8420-B3-005
	DAPT	Tocris	2634

Plate preparation

1 Plate preparation:

> Note
>
> This protocol has been used to differentiate dopaminergic neurons from hPSC adapted to feeder free culture systems. Our version of the protocol has been adapted from **Kim et al 2021; Cell Stem Cell 28, 343–355 e5, February 4, 2021** and **Piao et al. 2021; Cell Stem Cell 28, 217-229 e7, February 4, 2021**.

> Note
>
> We have used two alternative protocol variants with minor modifications which will be outlined at the respective steps as **SP** (Soldner Protocol) and **HP** (Hockemeyer Protocol).

> Note
>
> It is important to start the differentiation from pristine, undifferentiated feeder free cultures. For more details consult: https://doi.org/10.17504/protocols.io.b4mcqu2w

1.1 **Matrigel/Geltrex coating**: Prepare matrigel/geltrex (1:30) in cold DMEM/F12 or DPBS in a 15 ml tube as described by the manufacturer. Coat each well with 1-1.5 ml (6 well plate size) or 0.5 ml (12w plate size) of solution and incubate at 37 °C incubator for at least 00:30:00 to 1 hour. 30m

1.2 **Laminin coating**: Prepare laminin (2 µg/ml) in cold DPBS and coat each well of a 6-well plate with 1.5ml laminin solution (2 µg/ml) and incubate Overnight at 37 °C. 1h

1.3 **Poly-L-Ornithine (PLO) + Fibronectin (F) + Laminin (L) coating**: Prepare PLO (15 µg/ml) in sterile water and coat each well with 0.5-1 ml making sure it covers the entire surface. Incubate at 37 °C for 06:00:00 to overnight. The day after, wash each well with sterile water 4 times and add a new solution with fibronectin (1 µg/ml) and laminin (2 µg/ml) (in water) and incubate Overnight at 37 °C. Do not let the wells dry. 12h

1.4 **Poly-L-Ornithine (PLO) + Laminin (L) coating**: Prepare PLO (15 µg/ml) in sterile water and coat each well of a 12-well plate with 0.5-1 ml making sure it covers the entire 12h

surface. Incubate at 37 °C for 06:00:00 to overnight. The day after, wash each well with sterile water 2 times and add a new solution with laminin (2 µg/ml) (in cold DPBS) and incubate Overnight at 37 °C. Do not let the wells dry.

Media Preparation

2 Media recipes:

2.1 **Media A**: Neurobasal media + N2 supplement (1% vol/vol) + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + SHH (200 ng/ml) + CHIR99021 (0.7 µM) + LDN (250 nM) + SB431542 (10 µM).

2.2 **Media B**: Neurobasal media + N2 supplement (1% vol/vol) + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + SHH (200 ng/ml) + CHIR99021 (7.5 µM) + LDN (250 nM) + SB431542 (10 µM).

2.3 **Media C**: Neurobasal media + N2 supplement (1% vol/vol) + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + CHIR99021 (7.5 µM).

2.4 **Media D**: Neurobasal media + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + BDNF (20 ng/ml) + GDNF (20 ng/ml) + Ascorbic acid (200 µM) + Dibutyryl-cAMP (0.5 mM) + TGFβ3 (1ng/ml) + CHIR99021 (3 µM).

2.5 **Precursor splitting media**: Neurobasal media + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + BDNF (20 ng/ml) + GDNF (20 ng/ml) + Ascorbic acid (200 µM) + Dibutyryl-cAMP (0.5 mM) + TGFβ3 (1ng/ml).

2.6 **Maturation media**: Neurobasal media + B27 supplement without vitamin A (2% vol/vol) + L-Glutamine (2 mM) + Penicillin-Streptomycin (100U/ml) + BDNF (20 ng/ml) + GDNF (20 ng/ml) + Ascorbic acid (200 µM) + Dibutyryl-cAMP (0.5 mM) + TGFβ3 (1ng/ml) + DAPT (10 µM).

Dopaminergic neuron differentiation

1h 35m

3 Day 0: Dissociate hPSCs using accutase (00:10:00 - 37 °C). Quench dissociation by diluting the accutase solution with mTeSR-plus media + 10 µM Y-27632 and collect the cells into a 15ml conical tube. Spin the cells down at 105 rcf for 00:05:00. Remove the supernatant, resuspend the cells in mTeSR-plus media + 10 µM Y-27632 and count them.

15m

3.1 **SP**: Plate the cells at 400-600k/cm2 in media A + 10 µM Y-27632 onto geltrex coated plates (adjust cell number to the plate size being used).

3.2 **HP**: Plate the cells at 400-600k/well in a 6-well plate in mTeSR-plus + 10 µM Y-27632 onto matrigel coated plates.

4 Day 1-3: Change the media - media A (change daily or every other day as necessary).

5 Day 4 and day 6: Change the media- media B (change daily or every other day as necessary).

6 Day 7 and Day 9: Change the media - media C (change daily or every other day as necessary).

7 Day 10: Change the media - media D.

8 Day 11: Passage #1. Dissociate cells using accutase (approximately 00:10:00 - 37 °C but longer incubations may be necessary to properly detach the cells). Quench dissociation by diluting the accutase solution with 1 ml/well media D + 10 µM Y-27632. Collect cells in 15ml conical tubes and spin them down at 300 rcf - 00:05:00. Remove the supernatant, resuspend the cell in media D + 10 µM Y-27632. 15m

8.1 **SP:** Count the cells and plate them at 800k/cm2 in media D + 10 µM Y-27632 onto PLO+F+L coated plates.

8.2 **HP:** Split 1:2 in precursor splitting media + 10 µM Y-27632 onto laminin-coated plates.

9 Day 12-15: Change the media - maturation media (change daily or every other day as necessary).

10 Day 16: Passage #2. Dissociate cells using accutase (approximately 00:10:00 - 37 °C but longer incubations may be necessary to properly dissociate collect the cells). Quench dissociation by diluting accutase with 1 ml/well precursor splitting media + 10 µM Y-27632. Collect the cells in 15 ml conical tubes and spin them down at 15m

300 rcf - 00:05:00 . Remove the supernatant, resuspend the cell in precursor splitting media + 10 µM Y-27632 and count them.

10.1 **SP:** Plate the cells at 800k/cm2 in precursor splitting media + 10 µM Y-27632 onto PLO+F+L coated plates.

10.2 **HP:** Plate the cells at >1 million cells per well of 12-well plate in maturation media + 10 µM Y-27632 onto PLO+L coated plates.

11 Day 17-24: Change the media - maturation media (change daily or every other day as necessary).

12 Day 25: Passage #3.

12.1 **SP:** Dissociate cells using papain solution (00:30:00 - 37 °C). Inactivate papain solution using ovomucoid trypsin inhibitor (10mg/ml). Collect your cells in 15 ml conical tubes and spin them down at 300 rcf - 00:05:00 . Remove the supernatant, resuspend the cells in maturation media + 10 µM Y-27632 and count them. Plate the cells at 200-300k/cm2 in maturation media + 10 µM Y-27632 onto PLO+L+F coated plates. 35m

12.2 **HP:** Dissociate cells using accutase (approximately 00:10:00 - 37 °C is the usual standard but longer incubations may be necessary to properly detach the cells). Quench accutase by adding maturation media + 10 µM Y-27632. Collect your cells in 15 ml conical tubes and spin them down at 300 rcf - 00:05:00 . Remove the supernatant, resuspend the cells in maturation media + 10 µM Y-27632 and count them. Plate the cells at >1 million cells per well of 12-well plate in maturation media + 10 µM Y-27632 onto PLO+L coated plates. 15m

13 Day >26: Change media to maturation media (perform media changes every 2-3 days). Cells can be maintained in this media for several months or until experiment.