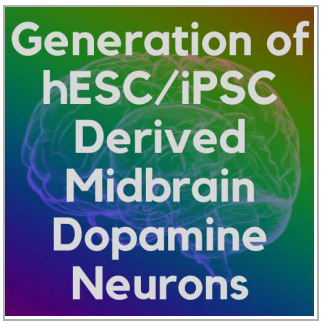

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WORKS FOR ME 1

Generation of hESC/iPSC Derived Midbrain Dopamine Neurons

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.kxygxpzjzl8j/v1

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ABSTRACT

This protocol details methods for generating Midbrain Dopamine Neuronds from hESC or iPSC sources.

DOI

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PROTOCOL CITATION

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KEYWORDS

generation, hESC, iPSC, midbrain , dopamine, neurons, differentiation , ASAPCRN

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References

CITATION

Xi J, Liu Y, Liu H, Chen H, Emborg ME, Zhang SC (2012). Specification of midbrain dopamine neurons from primate pluripotent stem cells.. Stem cells (Dayton, Ohio).

LINK

https://doi.org/10.1002/stem.1152

CITATION

Xiong M, Tao Y, Gao Q, Feng B, Yan W, Zhou Y, Kotsonis TA, Yuan T, You Z, Wu Z, Xi J, Haberman A, Graham J, Block J, Zhou W, Chen Y, Zhang SC (2020). Human Stem Cell-Derived Neurons Repair Circuits and Restore Neural Function.. Cell stem cell.

LINK

https://doi.org/pii:S1934-5909(20)30410-0.10.1016/j.stem.2020.08.014

SAFETY WARNINGS

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

BEFORE STARTING

Induction of the midbrain dopaminergic progenitors was carried out based on protocol described previously (Xi et al., 2012) with modification.

Pre-differentiation and Differentiation (Day 1-6)

1 Briefly, culture hESCs (1 day after passaging) on MEF feeder layer in the neural induction medium (NIM) (DMEM/F-12, 1X NEAA, 1X N2 supplement) supplemented with [M] 10 micromolar (μM) SB431542 and [M] 2 micromolar (μM) DMH-1 .

To pattern the differentiating cells to the midbrain FP progenitors, add [M] 500 nanogram per milliliter (ng/mL) SHH (C25II) and [M] 0.4 micromolar (μM) CHIR99021 to the cultures from day 1 till day 7.

Differentiation (Day 7-11)

2 On day 7, gently blow off individual colonies of neuroepithelial cells with a pipette and replate on mouse embryonic fibroblast feeder in the NIM containing [M] 1 micromolar (μM) SAG and [M] 100 nanogram per milliliter (ng/mL) SHH and [M] 0.4 micromolar (μM) CHIR99021 for additional 6 days (D7-12).

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Differentiation (Day 12-19)

3 On day 12, remove CHIR99021, reduce the following: [M] 20 nanogram per milliliter (ng/mL) SHH, [M] 0.5 micromolar (µM) SAG and [M] 100 nanogram per milliliter (ng/mL) FGF8b and add to the culture to expand the progenitors in suspension till day 19.

Differentiation (Day 19-31)

4 Then, keep [M] 20 nanogram per milliliter (ng/mL) SHH and [M] 20 nanogram per milliliter (ng/mL) FGF8b in the neural induction medium till transplantation/in vitro analysis at day 32.

8m

In Vitro Analysis (Day 32+)

8m

5 For in vitro analysis, dissociate neurospheres by incubation in Accutase at 37 °C for 00:03:00 - 00:05:00 on day 32 and plate onto glass coverslips that were previously coated with Matrigel.

6 Feed cells with Neural differentiation medium (Neurobasal™ Medium, 1 X N2 supplement, 1 X B27 supplement) (NDM) supplemented with brain-derived neurotrophic factor ([M] 10 nanogram per milliliter (ng/mL) BDNF), glial cell line derived neurotrophic factor ([M] 10 nanogram per milliliter (ng/mL) GDNF), ascorbic acid ([M] 200 micromolar (µM) AA), [M] 1 micromolar (µM) cAMP, transforming growth factor β3 ([M] 1 nanogram per milliliter (ng/mL)) and Compound E ([M] 0.1 micromolar (µM)).

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