Mar 31, 2023

Midbrain-like Organoids generation from hiPSCs

In 2 collections

DOI
dx.doi.org/10.17504/protocols.io.kqdg39d5qg25/v1

Hariam Raji[1], michela.deleidi[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli
ASAP

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg39d5qg25/v1

Protocol Citation: Hariam Raji, michela.deleidi 2023. Midbrain-like Organoids generation from hiPSCs. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg39d5qg25/v1**

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

Created: March 28, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 79544

Keywords: Midbrain-like Organoids, human induced pluripotent stem cells (hiPSCs), Matrigel embedding, ASAPCRN, like organoids generation from hipsc, pluripotent stem cell, induced pluripotent stem cell, like organoids generation, human midbrain, like organoid, hipsc, midbrain, cell

Abstract

In this protocol we describe the differentiation of human induced pluripotent stem cells (hiPSCs) into human midbrain-like organoids (hMLOs). This protocol has been developed based from several published protocols.

Attachments

PDF

366-820.pdf

517KB

Materials

Day 0-Medium composition:

	A	B
	DMEM F12 (w/o HEPES) /Neurobasal (1:1)	
	N2 0.5%	250 µL/50 mL
	B27 1%	500 µL/50 mL
	NEAA 1%	500 µL/50 mL
	B-MercaptoEtOH 0.1%	
	Heparin	1 µg/mL (100 mg/mL) 1:100.000
	SB431542	10 µM (10 mM) 1:1000
	Noggin	200 ng/mL (200 µg/mL) 1:1000 or (LDN 1:100.000)
	CHIR99021	0.7 µM (3 mM) 1:4285 11.7 µL/50 mL
	Rock Inhibitor (APOI)	50 µM (10 mM) 1:200

Day 4-Medium composition:

	A	B
	DMEM F12 (w/o HEPES)/Neurobasal (1:1)	
	N2 0.5%	250 µL/50 mL
	B27 1%	500 µL/50 mL
	NEAA 1%	500 µL/50 mL
	B-MercaptoEtOH 0.1%	
	Heparin	1 µg/mL (100 mg/mL) 1:100.000
	SB431542	10 µM (10 mM) 1:1000
	Noggin	200 ng/mL (200 µg/mL) 1:1000 or (LDN 1:100.000)
	CHIR99021	7.5 µM (3 mM) 1:400
	SHH-C25II	100 ng/mL (100 µg/mL) 1:1000
	FGF8	100 ng/mL (100 µg/mL) 1:1000
	PMA	1 µM (Stock: 1mM)

Day 7-Medium composition:

	A	B
	Neurobasal	
	N2	0.50%
	B27	1%
	Glutamax	1%
	NEAA	1%
	B-MercaptoEtOH	0.1%
	Insulin	2.5 µg/mL (5 mg/mL) 1:2000
	Laminin	200 ng/mL (100 µg/mL) 1:500
	SHH-C25II	100 ng/mL (100 µg/mL) 1:1000
	CHIR99021	7.5 µM (3 mM) 1:400
	FGF8	100 ng/mL (100 µg/mL) 1:10.000
	PMA	1 µM (Stock: 1 mM)

Final Differentiation Medium composition:

	A	B
	Neurobasal	
	N2	(250 µL/50 mL)
	B27	(500 µL/50 mL)
	Glutamax	1% (500 µL/50 mL)
	NEAA	1% (500 µL/50 mL)
	B-MercaptoEtOH	0.1% (50 µL/50 mL)
	BDNF	10 ng/mL (20 µg/mL) 1:2000
	GDNF	10 ng/mL (20 µg/mL) 1:2000
	Ascorbic Acid	100 µM (200 mM) 1:2000
	db-cAMP	125 µM (500 mM) 1:4000
	CHIR99021	7.5 µM (3 mM) 1:400

Cytokines and factors:

	A	B	C	D
	Company	Cat.Nr.	Name	Size
	ThermoScientific	12-587-010	B27	10 ml
	ThermoScientific	17502-048	N2	5 ml
	R&D System	3400-010-03	Laminin I	1 mg
	Corning	354230	Matrigel	10 ml
	Selleckchem	S1049	ApoI (Y-27632 2HCl)	
	AppliChem	A0455,1000	dbCAMP (C)	1 mg
	Sigma-Aldrich	540220-5MG	Purmorphamine (PMA)	
	R&D System	1614	SB 431542	10 mg
	PeproTech	450-02-500	BDNF	500 µg
	PeproTech	450-10-500	GDNF	500 µg
	PeproTech	100-25-500	FGF8	500 µg
	PeproTech	100-45-100	Sonic hedgehog	100 µg
	Sigma-Aldrich	A6964-100 ml	Accutase	100 ml
	Gibco	11320074	DMEM/F12 w/o HEPES	500 ml
	Gibco	21103049	Neurobasal Medium	500 ml
	Gibco	11140035	MEM NEAA	100 ml
	Gibco	35050038	Glutamaxx	100 ml
	Sigma	SML1046-5MG	CHIR 99021	3 mM
	Sigma	25556-4	L-Ascorbic Acid	200 mM
	ThermoScientific	12585014	Insulin, human recomb.	4 mg/ml
	Axon	1509	LDN 193189HCl	100 µM

B-27™ Supplement (50X) minus vitamin A **Thermo Fisher Scientific Catalog #**B-27™ Supplement (50X), minus vi

- N-2 Supplement (100X) **Thermo Fisher Catalog #**17502048
- Cultrex Stem Cell Qualified Laminin I Pathclear **R&D Systems Catalog #**3400-010-03
- Growth Factor Reduced (GFR) Matrigel® **Corning Catalog #**354230
- Y-27632 **Selleckchem Catalog #**S1049
- N6-2-O-Dibutyryl-Adenosine 35-Cyclophosphate Sodium Salt 1-hydrate BioChemica **Panreac AppliChem Catalog #** A0455,1000
- Purmorphamine **Merck Millipore (EMD Millipore) Catalog #**540220
- SB 431542 **R&D Systems Catalog #**1614
- BDNF **peprotech Catalog #**450-02-500
- GDNF **peprotech Catalog #**450-10-500
- FGF8 **peprotech Catalog #**100-25-500
- Sonic hedgehog **peprotech Catalog #**100-45-100
- Accutase® solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A6964
- DMEM/F-12 **Thermo Fisher Catalog #**11320074
- Neurobasal medium **Gibco - Thermo Fisher Scientific Catalog #**21103049
- MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Scientific Catalog #**11140035
- GlutaMAX **Gibco - Thermo Fisher Scientific Catalog #**35050038
- CHIR99021 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SML1046-5MG
- L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**255564
- Insulin, human recombinant, zinc solution **Thermo Fisher Catalog #**12585014
- LDN 193189 hydrochloride **Axon Medchem Catalog #**1509

Troubleshooting

Day 0

7m

1 Dissociate iPSC colonies to single cells with Accutase for 00:07:00 at 37 °C. 7m

2 Re-suspend cells in day0 medium and plate 8.000 cells/well in 96-Wells Plate U-round-Bottom Low Attachment.

Day 4

3 Carefully exchange the medium, without touching the EBs.

Day 7: Matrigel embedding

1d 0h 30m

4 Dilute Matrigel in a 3:2 ratio with day 4 medium (used as an embedding mixture).

5 Wash EBs in day 4 medium. Transfer and mix 5-8 EBs into the embedding mixture and plate onto a 6-well ultra-low-attachment plate.

6 Incubate for 00:30:00 at 37 °C and add day 7 medium. 30m

7 Incubate at least 24:00:00 between d7 and d8. 1d

Day 8

8 Add fresh differentiation medium without disrupting the embedded EBs.

9 After Day 8 change medium twice a week.

Day 10-13

10 At day 10 reduce CHIR to [M] 3.0 micromolar (µM) (1:1000).

Day 13-15

11 Reduce CHIR to [M] 0.7 micromolar (µM) . Remove CHIR from medium at day 15 and onwards.

Day 20-25

12 Manually dissociate organoids from Matrigel, using two surgical needles.

13 Place on orbital shaker after dissociation (80 rpm).