MAR 17, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvobr8xl4o/v1

Protocol Citation: Hariam Raji, michela.deleidi 2023. Midbrain-like Organoids generation from hiPSCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvobr8xl4o/v1

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

Created: Feb 21, 2022

Last Modified: Mar 17, 2023

PROTOCOL integer ID: 58488

Keywords: Midbrain-like Organoids, human induced pluripotent stem cells (hiPSCs), Matrigel embedding

Midbrain-like Organoids generation from hiPSCs

In 8 collections

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

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

Hariam Raji

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

366-820.pdf

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

	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
	A Neurobasal	B
	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	Apol (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

	A	B	C	D
	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 Catalog #21103049**

MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Scientific Catalog #11140035**

GlutaMAX **Gibco - Thermo Fisher 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 #1258501**

LDN 193189 hydrochloride **Axon Medchem Catalog #1509**

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).