



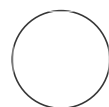
MAR 30, 2023

# Midbrain-like Organoids generation from hiPSCs

In 1 collection

Hariam Raji<sup>1</sup>, michela.deleidi<sup>1</sup>

<sup>1</sup>German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany



Federico Bertoli

OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.kqdg39d5qg25/v1](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>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

**Created:** Mar 28, 2023

**Last Modified:** Mar 30, 2023

**PROTOCOL integer ID:**  
 79544

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

## 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 (AP01)	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
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	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

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 $\mu$ M

⊗ 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-Dibutyl-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 #12585014**

## 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  3.0 micromolar ( $\mu\text{M}$ ) (1:1000).

## Day 13-15

- 11 Reduce CHIR to  0.7 micromolar ( $\mu\text{M}$ ) . 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 ↻).