Institut-Hôpital neurologique de Montréal

Montreal Neurological Institute-Hospital

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EDDU Protocols

Culture Reagents

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v2.0

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Antibiotic-Antimycotic

Gibco #15240-062

- Contains the antibiotics penicillin and st
  amphotericin B

*Select to go to the **Table of Contents** screen*

*Select to go to the **Materials** section*

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	MW/Concentration		Preparation instructio	Informa
As supplied/ stock solution	100x		• Solution	date of manufacture
Final solution (media)	iPSC culture; DA neuron, cortical neuron, and motor neuron differentiation	1x	• Dilute 1:100	4°C
Final solution (coating)	Laminin solution	1x	• Dilute 1:100	4°C

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Introduction

Introduction

Objectives

- This document provides key information for reagents used in culture media for:
  - Peripheral blood mononuclear cell (PBMC) reprogramming
  - Induced pluripotent stem cell (iPSC) culture
  - Mouse embryonic fibroblast (MEF) culture
  - Induction of dopaminergic (DA) neural progenitor cells (NPCs), cortical NPCs, and motor NPCs
  - NPC culture
  - Differentiation of dopaminergic (DA) neurons, cortical neurons, motor neurons, cholinergic neurons, sensory neurons, GABAergic neurons, astrocytes, microglia, and oligodendrocytes
  - 3D culture of forebrain, midbrain, and cerebral organoids
- The following information is provided for each reagent as supplied and for stock and final solutions, where applicable:
  - Molecular weight (MW) of solid powders or concentration of solutions
  - Preparation instructions
  - Storage conditions
  - Important information for handling and usage

Introduction

Technical and Safety Information

- This information in this document is not intended to replace the product information or material safety data sheet (MSDS) from the manufacturer. Refer to all documentation provided by the manufacturer prior to using any reagent.
- Follow all laboratory safety requirements as outlined by McGill University Environmental Health and Safety (EHS).
- All reagents in this document are for use in cell culture and should be kept sterile. All solutions should be prepared using sterile technique.
- For reagents supplied as solid powders, reconstitute the entire vial or bottle to prepare stock solutions when appropriate.
- Final solution information is provided for the most common application(s) and is subject to change depending on the application and in the event of protocol updates. Prepare final solutions as per the most recent version of the protocol.

Materials

Materials

Select a culture reagent to go to its screen. **Culture Reagents A–H**

- Activin-A
- Antibiotic-Antimycotic
- Ascorbic acid (AA)
- B-27 supplement
- B-27 supplement, minus Vitamin A
- Biotin
- Bone morphogenetic protein 4 (BMP4)
- Bone morphogenetic protein 9 (BMP9)/GDF-2
- Bovine serum albumin (BSA)
- Brain-derived neurotrophic factor (BDNF)
- CD200
- CHIR-99021
- Ciliary neurotrophic factor (CNTF)
- Compound E
- CX3CL1
- DAPT
- Dexamethasone
- Dibutyryl-cAMP (db-cAMP)
- DMEM/F12
- DMEM
- Dorsomorphin
- Dorsomorphin homologue 1 (DMH1)
- Embryonic stem-cell fetal bovine serum (FBS)
- Epidermal growth factor (EGF)
- Erythropoietin (EPO)
- Essential 8 supplement
- Essential 8 basal media
- Fetal bovine serum (FBS)
- Fibroblast growth factor–basic (FGF-b)/FGF-2
- Fibroblast growth factor 8 (FGF-8)
- 5-Fluoro-2′-deoxyuridine
- Gentle Cell Dissociation Reagent
- Glial-derived neurotrophic factor (GDNF)
- GlutaMAX-I
- Heparin

Materials

Select a culture reagent to go to its screen. **Culture Reagents: I–P**

- Insulin
- Insulin-like growth factor 1 (IGF-1)
- Insulin-transferrin-sodium selenite supplement (ITS)
- Interleukin-3 (IL-3)
- Interleukin-6 (IL-6)
- Interleukin-34 (IL-34)
- Knockout DMEM/F12
- Knockout serum replacement
- Laminin (Invitrogen)
- Laminin (Sigma)
- L-Ascorbic acid 2-phosphate (AA2P)
- LDN193189
- L-Glutamine
- Lithium chloride (LiCl)
- Macrophage colony stimulating factor (M-CSF)
- Matrigel growth factor reduced (GFR) basement membrane matrix
- Matrigel human embryonic stem cell (hESC)–qualified matrix
- MEM nonessential amino acid (NEAA) solution
- 2-mercaptoethanol (β-mercaptoethanol [BME]; Gibco #21985023)
- 2-mercaptoethanol (β-mercaptoethanol [BME]; Merck #8057400005)
- Mitomycin C
- mTeSR1 5x supplement
- mTeSR1 basal media
- N-2 supplement
- β-Nerve growth factor (β-NGF)
- Neurobasal (NB) media
- Neurotrophin-3 (NT3)
- Noggin
- Penicillin-Streptomycin
- Platelet-derived growth factor–AA (PDGF-AA)
- Phosphate-buffered saline (PBS)
- Poly-L-ornithine (PO)
- Polyvinyl alcohol (PVA)
- Purmorphamine

Materials

Select a culture reagent to go to its screen. **Culture Reagents: Q–Z**

- Retinoic acid (RA)
- SB431542
- Smoothened Agonist (SAG)
- Sodium butyrate
- Sonic hedgehog (SHH)
- Sonic hedgehog (SHH; C24II)
- Stem cell factor (SCF)
- Stemline II hematopoietic stem cell expansion medium
- StemPro Accutase Cell Dissociation Reagent
- Thiazovivin
- 1-Thioglycerol
- Thrombopoietin (TPO)
- Transforming growth factor–β3 (TGF-β3)
- 3,3′,5-Triiodo-L-thyronine (T3)
- Valproic acid (VPA)
- XAV939
- Y-27632

Culture Reagents: A–H

Activin A

Peprotech #120-14

- Regulates cell proliferation and differentiation and promotes neuronal survival

		MW/Concentration		Preparation instructions	Storage	Important information
As supplied		26.0 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		12.5 µg/mL		• Dissolve 100 ug in 8 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation		12.5 ng/mL	• Dilute stock solution 1:1000	4°C	

Antibiotic-Antimycotic

Gibco #15240-062

- Contains the antibiotics penicillin and streptomycin and the antifungal agent amphotericin B

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	100x		• Solution	–20°C for up to 1 year from date of manufacture	
Final solution (media)	iPSC culture; DA neuron, cortical neuron, and motor neuron differentiation	1x	• Dilute stock solution 1:100	4°C	
Final solution (coating)	Laminin solution	1x	• Dilute stock solution 1:100	4°C	

Ascorbic acid (AA)

Sigma #A5960

- An enzymatic cofactor and antioxidant

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	176.12 g/mol		• Powder	Room temperature	• May darken in storage
Stock solution	200 mM		• Dissolve 176.12 mg in 5 mL sterile ddH_2O • Filter solution • Prepare 200-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron or cortical neuron differentiation	200 µM	• Dilute stock solution 1:1000	4°C	
	Motor neuron differentiation, midbrain organoid culture	100 µM	• Dilute stock solution 1:2000	4°C	

B-27 supplement

Gibco #17504044

- Supplement that supports the neuronal growth and viability

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	50x		• Solution	–20°C for 12 months from date of manufacture	• Protect from light
Final solution (media)	DA neuron, cortical neuron, and astrocyte differentiation	1x	• Dilute stock solution 1:50	4°C	• Protect from light
	Motor neuron differentiation, cerebral organoid culture	0.5x	• Dilute stock solution 1:100	4°C	• Protect from light

B-27 supplement, minus Vitamin A

Gibco #12587010

- Supplement that is ideal for the cultivation of neural progenitor and stem cells, either as organoids in suspension or in adherent monolayer culture, without inducing differentiation

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	50x		• Solution	–20°C for 12 months from date of manufacture	• Protect from light
Final solution (media)	Forebrain and midbrain organoid culture	1x	• Dilute stock solution 1:50	4°C	• Protect from light
	Cerebral organoid culture	0.5x	• Dilute stock solution 1:100	4°C	• Protect from light

Biotin

Sigma #B4639

- Essential vitamin that is important for amino acid and energy metabolism and fatty acid synthesis

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	244.31 g/mol		• Powder	4°C	
Stock solution	100 µg/mL		• Dissolve 10 mg in 1 mL 1N NaOH • Add 100 µL of solution to 9.9 mL 1x PBS • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Oligodendrocyte differentiation	100 ng/mL	• Dilute stock solution 1:1000	4°C	

Bone morphogenetic protein 4 (BMP4)

GenScript #Z02750

- Regulates growth, differentiation, chemotaxis, and apoptosis of various cell types

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	~13.3 kDa		• Powder; briefly centrifuge vial before opening	–20°C	• Stable at 4°C but should be kept at –20°C for long-term storage
Stock solution	50 µg/mL		• Dissolve 100 mg in 2 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Stable at 4°C for up to 1 week • Avoid repeated freeze-thaw cycles
Final solution (media)	Microglia differentiation	50 ng/mL	• Dilute stock solution 1:1000	4°C	

Bone morphogenetic protein 9 (BMP9)/GDF-2

Peprotech #120-07

- Regulates cell differentiation and survival

		MW/Concentration		Preparation instructions	Storage	Important information
As supplied		24.1 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL		• Dissolve 200 µg in 20 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Cholinergic neuron differentiation	10 ng/mL		• Dilute stock solution 1:1000	4°C	

Bovine serum albumin (BSA)

Invitrogen #15260-037

- Supports growth of human hematopoietic progenitor cells in serum-free media formulations

	 MW/Concentration		 Preparation instructions	 Storage	 Important information
As supplied/ stock solution	75 mg/mL in PBS		• Liquid	–20°C	
Final solution (media)	DA NPC and cortical NPC induction	1 mg/mL	• Dilute stock solution 1:75	4°C	

Brain-derived neurotrophic factor (BDNF)

Peprotech #450-02

- Neurotrophic growth factor that supports neuronal proliferation and survival

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		27.0 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		20 µg/mL	• Dissolve 500 µg in 25 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA and cortical neuron differentiation, forebrain organoid culture	20 ng/mL	• Dilute stock solution 1:1000	4°C	
	Motor neuron differentiation, midbrain organoid culture	10 ng/mL	• Dilute stock solution 1:2000	4°C	

CD200

Novoprotein #C311

- Immunoregulatory protein

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	30 kDa	• Powder; briefly centrifuge vial before opening	–20°C	• Stable at room temperature for up to 3 weeks
Stock solution	100 µg/mL	• Dissolve 200 µg in 2 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C for up to 3 months	• Do not mix by vortex or pipetting • Stable at 4°C for 2 to 7 days • Minimize freeze-thaw cycles
Final solution (media)	Microglia differentiation	100 ng/mL • Dilute stock solution 1:1000	4°C	

CHIR-99021

Selleckchem #S2924

- Inhibits GSK-3α/β leading to activation of GSK and insulin signaling

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		501.4 g/mol	• Powder; briefly centrifuge vial before opening	–20°C for up to 3 years	
Stock solution		3 mM	• Dissolve 25 mg in 16.607 mL DMSO • Prepare 200-µL aliquots in 1.5-mL tubes	–80°C for up to 2 years	
Final solution (media)	DA NPC and cortical NPC induction; motor NPC induction (step 1 and 3 media)	3 µM	• Dilute stock solution 1:1000	4°C	
	Motor NPC induction (step 2 media)	1 µM	• Dilute stock solution 1:3000	4°C	
	Midbrain organoid culture	0.8 µM	• Dilute stock solution 1:3750	4°C	

Ciliary neurotrophic factor (CNTF)

Peprotech #450-13

- Neural factor that supports neuronal survival

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	22.8 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	10 µg/mL		• Dissolve 250 µg in 25 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Motor neuron and astrocyte differentiation	10 ng/mL	• Dilute stock solution 1:1000	4°C	

Compound E

STEMCELL Technologies #73954

- Inhibits γ-secretase and Notch processing

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	490.5 g/mol		• Powder; briefly centrifuge vial before opening	–20°C for up to 12 months For long-term storage, store with a desiccant	• Protect from light
Stock solution	0.1 mM		• Dissolve 1 mg in 20.388 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–20°C	• Avoid repeated freeze-thaw cycles • Protect from light
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	0.1 µM	• Dilute stock solution 1:1000	4°C	• Protect from light

CX3CL1

Peprotech #300-31

- Chemokine that chemoattracts specific cell types, including microglia cells

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	8.5 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	100 µg/mL		• Dissolve 500 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation	100 ng/mL	• Dilute stock solution 1:1000	4°C	

DAPT

Selleckchem #S2215

- Inhibits γ-secretase

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	432.46 g/mol		• Powder; briefly centrifuge vial before opening	–20°C for up to 3 years	
Stock solution	10 mM		• Dissolve 10 mg in 2.312 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C for up to 2 years	
Final solution (media)	DA neuron and sensory neuron differentiation	10 µM	• Dilute stock solution 1:1000	4°C	

Dexamethasone

Sigma #D4902

- Activates glucocorticoid receptor signaling

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	392.46 g/mol		• Powder; briefly centrifuge vial before opening	4°C	
Stock solution	10 mM		• Dissolve 25 mg in 6.37 mL DMSO • Prepare 20-µL aliquots in 0.7-mL tubes	–80°C	• Avoid repeated freeze-thaw cycles
Final solution (media)	PBMC reprogramming	1 µM	• Dilute stock solution 1:10,000	4°C	

Dibutyryl-cAMP (db-cAMP)

Carbosynth #ND07996

- cAMP analog that actives cAMP-dependent kinases

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		491.37 g/mol	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		0.5 M	• Dissolve 1 g in 4.07 mL sterile ddH_2O • Prepare 200-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron and cortical neuron differentiation	0.5 mM	• Dilute stock solution 1:1000	4°C	
	Midbrain organoid culture	125 µM	• Dilute stock solution 1:4000	4°C	

Dorsomorphin

Tocris #3093

- Inhibits AMPK and type I BMP receptors

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		472.4 g/mol	• Powder; briefly centrifuge vial before opening	–20°C	• Product is hygroscopic. Desiccate upon arrival • Can be stored at room temperature for up to 6 months
Stock solution		20 mM	• Dissolve 10 mg in 4.237 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Can be stored at –20°C for up to 1 month
Final solution (media)	NPC induction	2 µM	• Dilute stock solution 1:10,000	4°C	
	Forebrain organoid culture	10 µM	• Dilute stock solution 1:2000	4°C	

Dorsomorphin homolog 1 (DMH1)

Selleckchem #S7146

- Inhibits ALK2, a type 1 BMP receptor

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	380.44 g/mol		• Powder; briefly centrifuge vial before opening	–20°C for up to 3 years	
Stock solution	4 mM		• Dissolve 25 mg in 16.425 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C for up to 2 years	
Final solution (media)	Motor neuron and astrocyte differentiation	2 µM	• Dilute stock solution 1:2000	4°C	

DMEM/F12 media

Gibco #10565-018

- Basal media for supporting growth of mammalian cells

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	1x		• Solution	4°C for up to 12 months from date of manufacture	• Protect from light
Final solution (coating)	Laminin solution	1x	• Add Antibiotic-Antimycotic and laminin as per protocol	4°C	• Protect from light
Final solution (media)	iPSC culture; DA neuron, cortical neuron, motor neuron, and astrocyte differentiation; cerebral organoid culture	1x	• Add supplements as per protocol	4°C	• Protect from light

DMEM

Wisent Bioproducts #319-005-CL

- Basal media for supporting growth of mammalian cells

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	1x		• Solution	4°C	
Final solution (media)	MEF culture	1x	• Add supplements as per protocol	4°C	

Embryonic stem-cell fetal bovine serum (FBS)

Gibco #10439024

- Sustains undifferentiated cellular morphology of embryonic stem cells

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	100%		• Solution	–20°C	
Final solution (media)	Cerebral organoid culture	3%	• Add 3 mL to 100 mL culture media	4°C	

Epidermal growth factor (EGF)

Peprotech #AF-100-15

- Growth factor that stimulates growth of epidermal and epithelial cells

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		6.2 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL	• Dissolve 1 mg in 100 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron, cortical neuron, and astrocyte differentiation*	10 ng/mL	• Dilute stock solution 1:1000	4°C	
	Astrocyte differentiation*	20 ng/mL	• Dilute stock solution 1:500	4°C	

*Final solution concentration for astrocyte differentiation depends on protocol.

Erythropoietin (EPO)

Peprotech #100-64

- Hormone that stimulates proliferation and differentiation of erythroid progenitor cells

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	150 U/µg*		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	2000 U/µL		• Dissolve 13.3 µg in 1 mL 1x PBS • Prepare 50-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming	2 U/mL	• Dilute stock solution 1:1000	4°C	

*1 U is equivalent to 6.7 ng.

Essential 8 supplement

Gibco #A1517001 (component #A1517101)

- Supplement for Essential 8 basal medium

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	50x		• Solution	–20°C for up to 12 months from date of manufacture	• Store in non-frost-free freezer • Do not re-freeze thawed solution
Final solution (media)	iPSC culture	1x	• Thaw Essential 8 50x supplement for about 1 hour at room temperature or overnight at 4°C. Mix gently • Add entire bottle (10 mL) of Essential 8 50x supplement to 500 mL of Essential 8 basal media • Prepare 40-mL aliquots in 50-mL conical tubes, seal with Parafilm, and store at –20°C	4°C for up to 2 weeks –20°C for up to 6 months	• Do not thaw/warm supplement or complete media in a 37°C water bath • Once supplement is thawed, use immediately or store at 4°C for up to 2 days • Thaw complete media aliquots overnight at 4°C. Do not refreeze aliquots after thawing • Warm complete media at room temperature

Essential 8 basal media

Gibco #A1517001 (component #A1516901)

- Xeno-free and feeder-free medium used for the growth and expansion of human iPSCs

	MW/Concentration	Preparation instructions	Storage	Important instructions
As supplied	1x	• Solution	4°C for up to 12 months from date of manufacture	• Protect from light
Final solution (media)	iPSC culture 1x	• Thaw Essential 8 50x supplement for about 1 hour at room temperature or overnight at 4°C. Mix gently • Add entire bottle (10 mL) of Essential 8 50x supplement to 500 mL of Essential 8 basal media • Prepare 40-mL aliquots in 50-mL conical tubes, seal with Parafilm, and store at –20°C	4°C for up to 2 weeks –20°C for up to 6 months	• Do not thaw/warm supplement or complete media in a 37°C water bath • Once supplement is thawed, use immediately or store at 4°C for up to 2 days • Thaw complete media aliquots overnight at 4°C. Do not refreeze aliquots after thawing • Warm complete media at room temperature

Fetal bovine serum (FBS)

ThermoFisher #12484028

- Supplement for culture media
- Cryopreservation media

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied/ stock solution	100%		• Solution	–20°C	
Final solution (cryo-preservation)	iPSCs, DA NPCs, cortical NPCs, and motor NPCs	90%	• Add DMSO at 10%	Room temperature	
Final solution (media)	MEF culture	10%	• Dilute stock solution 1:10	4°C	

Fibroblast growth factor–basic (FGF-b)/FGF-2

Peprotech #100-18B

- Growth factor that promotes proliferation and differentiation of a variety of cell types

		MW/Concentration		Preparation instructions	Storage	Important information
As supplied		17.2 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL		• Dissolve 1 mg in 100 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming; DA neuron, cortical neuron, and astrocyte differentiation*		10 ng/mL	• Dilute stock solution 1:1000	4°C	
	Astrocyte differentiation*		20 ng/mL	• Dilute stock solution 1:500	4°C	
	Cerebral organoid culture		4 ng/mL	• Dilute stock solution 1:2500	4°C	

*Final solution concentration for astrocyte differentiation depends on protocol.

Fibroblast growth factor 8 (FGF-8)

Peprotech #100-25

- Growth factor that promotes cell proliferation and differentiation

	 MW/Concentration		 Preparation instructions	 Storage	 Important information
As supplied	22.5 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	100 µg/mL		• Dissolve 500 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	NPC induction and midbrain organoid culture	100 ng/mL	• Dilute stock solution 1:1000	4°C	

5-Fluoro-2′-deoxyuridine

Sigma #F0503

- Antineoplastic agent that inhibits thymidylate synthetase

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	246.19 g/mol		• Powder; briefly centrifuge vial before opening	Room temperature	
Stock solution	100 mM		• Dissolve 100 mg in 406 µL sterile ddH_2O • Prepare 20-µL aliquots in 0.7-mL tubes	–80°C	• Stable at 4°C for up to 2 weeks • Protect from light
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	1 µM	• Dilute stock solution 1:100,000	4°C	• Protect from light

Gentle Cell Dissociation Reagent

STEMCELL Technologies #07174

- Enzyme-free reagent for dissociation of human embryonic stem cells or human (iPSCs) cells into cell aggregates for routine passaging or into a single-cell suspension

	 MW/Concentration	 Preparation instructions	 Storage	 Important instructions
As supplied	iPSCs, DA NPCs, cortical NPCs, and motor NPCs	1x	• Solution	Room temperature

Glial-derived neurotrophic factor (GDNF)

Peprotech #450-10

- Neurotrophic factor that promotes dopamine uptake and survival and morphological differentiation of midbrain neurons

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		30.4 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		20 µg/mL	• Dissolve 500 µg in 25 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron and cortical neuron differentiation	20 ng/mL	• Dilute stock solution 1:1000	4°C	
	Midbrain organoid culture	10 ng/mL	• Dilute stock solution 1:2000	4°C	

GlutaMAX-I

Gibco #35050-061

- Supplement for adherent and suspension culture of mammalian cells that is an alternative to L-glutamine, with increased stability that improves cell health

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	100x (200 mM in 0.85% NaCl)		• Solution	Room temperature for up to 24 months from date of manufacture	
Final solution (media)	DA NPC and cortical NPC induction; motor neuron differentiation; midbrain and cerebral organoid culture	1x	• Dilute stock solution 1:100	4°C	

Heparin

Sigma #H3149

- Enhances the antithrombin-mediated inactivation of proteases in the coagulation pathway

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		Mixture of polyanion chains of MW 6–30 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		20 mg/mL	• Dissolve 477 mg in 23.85 mL 1x PBS • Prepare 50-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Astrocyte differentiation	2 µg/mL	• Dilute stock solution 1:10,000	4°C	
	Midbrain and cerebral organoid culture	1 µg/mL	• Dilute stock solution 1:20,000	4°C	

Culture Reagents: I–P

Insulin

Sigma #I2643

- Regulates cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits breakdown of glycogen, protein, and fat

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		5807.57 g/mol	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		5 mg/mL	• Dissolve 50 mg in 10 mL sterile ddH_2O containing 0.1% BSA • Prepare 200-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Midbrain organoid culture	2.5 µg/mL	• Dilute stock solution 1:2000	4°C	
	Cerebral organoid culture	1.25 µg/mL	• Dilute stock solution 1:4000	4°C	

Insulin-like growth factor 1 (IGF-1)

Peprotech #100-11

- Growth factor that stimulates proliferation and differentiation of various cell types

	 MW/Concentration		 Preparation instructions	 Storage	 Important information
As supplied	7.6 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	10 µg/mL		• Dissolve 1 mg in 100 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming, motor neuron differentiation	10 ng/mL	• Dilute stock solution 1:1000	4°C	

Insulin-transferrin-sodium selenite supplement (ITS)

Gibco #41400045

- Insulin promotes glucose and amino acid uptake, lipogenesis, intracellular transport, and protein and nucleic acid synthesis. Transferrin is an iron carrier and may reduce toxic levels of oxygen radicals and peroxide. Selenium, as sodium selenite, is a cofactor for glutathione peroxidase and other proteins, and an antioxidant

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	100x		• Solution	4°C for up to 18 months from date of manufacture	
Final solution (media)	PBMC reprogramming, microglia differentiation	1x	• Dilute stock solution 1:100	4°C	• Protect from light

Interleukin-3 (IL-3)

Peprotech #200-03

- Hematopoietic growth factor that promotes the survival, differentiation and proliferation of specific types of committed progenitor cells, including those of the erythroid lineage

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		15.0 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL	• Dissolve 1 mg in 100 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming	10 ng/mL	• Dilute stock solution 1:1000	4°C	

Interleukin-6 (IL-6)

Peprotech #200-06

- Cytokine with diverse biological functions

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	20.9 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	50 µg/mL		• Dissolve 500 µg in 10 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation	50 ng/mL	• Dilute stock solution 1:1000	4°C	

Interleukin-34 (IL-34)

Peprotech #200-34

- Ligand for colony-stimulating factor-1 receptor (CSF1R)

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	52.5 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	100 µg/mL		• Dissolve 500 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation	100 ng/mL	• Dilute stock solution 1:1000	4°C	

KnockOut serum replacement

Gibco #10828028

- Supplement for KnockOut DMEM/F12 basal media

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied		100%	• Solution • Prepare 10-mL aliquots in 15-mL tubes	–20°C for up to 18 months from date of manufacture	• Avoid repeated freeze-thaw cycles • Can be stored at 4°C for up to 4 weeks • Protect from light
Final solution (media)	PBMC reprogramming, iPSC culture, cerebral organoid culture	20%	• Thaw aliquot of KnockOut serum replacement overnight at 4°C • Add 20 mL KnockOut serum replacement to 100 mL KnockOut DMEM/F12 • Add supplements as per protocol	4°C for up to 10 days	• Do not thaw/warm serum replacement or complete media in a 37°C water bath • Warm only the volume of complete media required for that day's use • Protect complete media from light

KnockOut DMEM/F12

Gibco #12660012

- Low osmolality medium without L-glutamine or HEPES buffer for the growth of human embryonic stem cells and iPSCs

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	1x		• Solution	4°C	
Final solution (media)	PBMC reprogramming, iPSC culture	1x	• Thaw aliquot of KnockOut serum replacement overnight at 4°C • Add 20 mL KnockOut serum replacement to 100 mL KnockOut DMEM/F12 • Add supplements as per protocol	4°C for up to 10 days	• Do not thaw/warm serum replacement or complete media in a 37°C water bath • Warm only the volume of complete media required for that day's use • Protect complete media from light

Laminin

Invitrogen #23017-015

- Extracellular matrix protein that supports adhesion, proliferation, and differentiation of many cell types

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	0.5–2.0 mg/mL* in 50 mM Tris-HCl (pH 7.4), 0.15 M NaCl		• Solution • Thaw solution slowly at 4°C • Prepare 100-µL aliquots in 0.7-mL polypropylene microcentrifuge tubes	–80°C	• If frozen solution is warmed too quickly, laminin may form a gel and cannot be reactivated for use • Do not freeze-thaw repeatedly
Final solution (coating)	DA neuron, cortical neuron, motor neuron, and astrocyte differentiation	5 µg/mL	• Dilute stock solution to 5 µg/mL in DMEM/F12	4°C for up to 2 weeks	• Do not store for more than 2 weeks
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	1 µg/mL	• Dilute stock solution to 1 µg/mL	4°C for up to 2 weeks	• Do not store for more than 2 weeks

*Verify exact concentration on tube label.

Laminin

Sigma #L2020

- Extracellular matrix protein that supports adhesion, proliferation, and differentiation of many cell types

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	~1 mg/mL* in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl		• Solution • Thaw solution slowly at 4°C • Prepare 100-µL aliquots in 0.7-mL polypropylene microcentrifuge tubes	–80°C	• If the frozen solution is warmed too quickly, laminin may form a gel and cannot be reactivated for use
Final solution (coating)	DA NPC and cortical NPC induction; DA neuron, cortical neuron, motor neuron, and astrocyte differentiation	5 µg/mL	• Dilute stock solution to 5 µg/mL in DMEM/F12	4°C for up to 2 weeks	• Do not use if discoloration or web formations appear on coated surfaces
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	1 µg/mL	• Dilute stock solution to 1 µg/mL	4°C for up to 2 weeks	• Do not store for more than 2 weeks
	Midbrain organoid culture	200 ng/mL	• Dilute stock solution to 200 ng/mL	4°C for up to 2 weeks	• Do not store for more than 2 weeks

*Verify exact concentration on tube label.

L-Ascorbic acid 2-phosphate (AA2P; sesquimagnesium salt hydrate)

Sigma #A8960

- Long-acting ascorbic acid derivative that stimulates collagen expression and formation

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	289.54 g/mol		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	32 mg/mL		• Dissolve 32 mg in 1 mL sterile ddH_2O • Prepare 100-µL aliquots in 0.7-mL tubes	4°C	
Final solution (media)	PBMC reprogramming	64 µg/mL	• Dilute stock solution 1:500	4°C	

LDN193189

Sigma #SML0559

- Dorsomorphin derivative that inhibits ALK2 and ALK3

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		406.48 g/mol	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		1 mM	• Dissolve 5 mg in 12.3 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Astrocyte differentiation	0.1 µM	• Dilute stock solution 1:10,000	4°C	

L-Glutamine

Wisent Bioproducts #609-065-EL

- Amino acid required for cell culture involved in the formation of purine and pyrimidine nucleotides, amino sugars, glutathione, L-glutamate, other amino acids; protein synthesis; and glucose production

	 MW/Concentration		 Preparation instructions	 Storage	 Important information
As supplied/ stock solution	200 mM		• Solution	–20°C	
Final solution (media)	PBMC reprogramming, MEF culture	2 mM	• Dilute stock solution 1:100	4°C	

Lithium chloride (LiCl)

Sigma #L7026

- Greatly enhances the generation of iPSCs

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	8 M in H_2O		• Solution	4°C	
Stock solution	2 M		• Dilute in sterile ddH_2O to achieve 2 M • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	4°C	
Final solution (media)	PBMC reprogramming	2 mM	• Dilute stock solution 1:1000	4°C	

Macrophage colony stimulating factor (M-CSF)

Peprotech #300-25

- Hematopoietic factor that regulates cellular proliferation, differentiation, and survival in blood monocytes, tissue macrophages, and their respective progenitor cells

		MW/Concentration		Preparation instructions	Storage	Important information
As supplied		36.8 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		25 µg/mL		• Dissolve 500 µg in 20 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation	25 ng/mL		• Dilute stock solution 1:1000	4°C	

Matrigel growth factor reduced (GFR) basement membrane matrix

Corning #356230

- Solubilized basement membrane extract containing laminin (a major component), collagen IV, heparin sulfate proteoglycans, entactin/nidogen, and a number of growth factors

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	1x in DMEM, 50 µg/mL gentamycin		• Solution	–20°C	• Do not store in frost-free freezer
Final solution (coating)	Midbrain and cerebral organoid culture	1x	• Thaw stock solution overnight at 4°C on ice	4°C	• Chill pipette tips at –20°C for 3 hours • Use entire bottle after thawing

Matrigel human embryonic stem cell (hESC)–qualified matrix

Corning Millipore #354277

- Soluble basement membrane extract containing laminin, collagen IV, entactin and heparan sulfate, and proteoglycan

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied/ stock solution	100x* in DMEM, 50 µg/mL gentamycin	• Solution • Thaw overnight at 4°C on ice • Prepare 150-µL aliquots in pre-chilled 0.7-mL tubes on ice	• As supplied: –20°C • Aliquots: –80°C	• Chill tubes and pipette tips at –20°C for 3 hours • Prepare one time–use aliquots to minimize freeze-thaw cycles • Do not store in frost-free freezer
Final solution (coating)	iPSC culture, motor neuron and astrocyte differentiation 1x	• Thaw aliquot of stock solution on ice • Dilute stock solution 1:100	4°C	• Use immediately after preparation or store at –20°C for up to 1 week. Thaw on ice and use immediately. Do not re-freeze

*Exact concentration on tube label.

2-Mercaptoethanol (β-mercaptoethanol [BME])

Gibco #21985023

- Reducing agent used in culture media to prevent toxic levels of oxygen radicals

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	55 mM in Dulbecco's phosphate-buffered saline (DPBS)		• Solution	4°C for 36 months from date of manufacture	
Final solution (media)	Forebrain organoid culture	0.1 nM	• Prepare serial dilutions of stock solution in sterile ddH_2O (e.g. 1:1000 to 1:50,000) • Dilute appropriate serial dilution to achieve 0.1 nM	4°C	• The 1:1000 serial dilution of the stock solution (55 µM) may be stored at 4°C for up to 3 months when used for the same batch of organoids. Prepare fresh serial dilutions for a new batch of organoids.

2-Mercaptoethanol (β-mercaptoethanol [BME])

Merck #8057400005

- Reducing agent used in culture media to prevent toxic levels of oxygen radicals

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied/ stock solution		78.12 g/mol (14.25 mM)	• Solution	Room temperature	
Final solution (media)	Midbrain organoid culture	0.00035%	• Dilute stock solution to 0.00035%	4°C	
	Cerebral organoid culture	0.00035% or 0.0007%	• Dilute stock solution to 0.00035% or 0.0007%, as per protocol	4°C	

MEM nonessential amino acid (NEAA) solution

Wisent #321-011-EL

- Supplement to increase cell growth and viability

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied/ stock solution		100x	• Solution	4°C	
Final solution (media)	PBMC reprogramming; DA NPC and cortical NPC induction; DA neuron, cortical neuron, and astrocyte differentiation; midbrain and cerebral organoid culture*	1x	• Dilute stock solution 1:100	4°C	
	Cerebral organoid culture*	0.5x	• Dilute stock solution 1:200	4°C	

*Final solution concentration for cerebral organoid culture depends on cerebral organoid stage, as per protocol.

Mitomycin C

Sigma #M4287

- Antibiotic and double-stranded DNA alkylating agent that inhibits cell proliferation

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	334.33 g/mol		• Powder; briefly centrifuge vial before opening	4°C	
Stock solution	1 mg/mL		• Dissolve 2 mg in 2 mL sterile ddH_2O • Prepare 50-µL aliquots in 0.7-mL tubes	–80°C	• Can be stored at 4°C for up to 1 week (pH 6–9) • If a precipitate forms, prepare a fresh stock solution • Protect from light
Final solution (media)	DA neuron and cortical neuron differentiation	1 µg/mL	• Dilute stock solution 1:1000	4°C	• Protect from light

mTeSR1 5x supplement

STEMCELL Technologies #85850 (component #85852)

- Supplement for mTeSR1 basal media

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	5x		• Solution	–20°C for up to 3 months	
Final solution (media)	iPSC culture	1x	• Thaw mTeSR1 5x supplement overnight at 4°C. Mix thoroughly • Add 100 mL mTeSR1 5x supplement to 400 mL mTeSR1 basal media to obtain 500 mL of complete media. Mix well • Prepare 40-mL aliquots in 50-mL conical tubes, seal with Parafilm, and store at –20°C	4°C for up to 2 weeks –20°C for up to 6 months	• Do not thaw/warm supplement or complete media in a 37°C water bath • Once supplement is thawed, use immediately or store at 4°C for up to 2 days • Thaw complete media aliquots overnight at 4°C. Do not refreeze aliquots after thawing • Warm complete media at room temperature

mTeSR1 basal media

STEMCELL Technologies #85850 (component #85851)

- Feeder-free cell culture media for human embryonic stem cells and iPSCs

	MW/Concentration		Preparation instructions	Storage	Important instructions
As supplied	1x		• Solution	4°C	
Final solution (media)	iPSC culture	1x	• Thaw mTeSR1 5x supplement overnight at 4°C. Mix thoroughly • Add 100 mL mTeSR1 5x supplement to 400 mL mTeSR1 basal media to obtain 500 mL of complete media. Mix well • Prepare 40-mL aliquots in 50-mL conical tubes, seal with Parafilm, and store at –20°C	4°C for up to 2 weeks –20°C for up to 6 months	• Do not thaw/warm supplement or complete media in a 37°C water bath • Once supplement is thawed, use immediately or store at 4°C for up to 2 days • Thaw complete media aliquots overnight at 4°C. Do not refreeze aliquots after thawing • Warm complete media at room temperature

N-2 supplement

Gibco #17502048

- Supplement that promotes neuronal cell growth

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied/ stock solution		100x	• Solution	–20°C	• Protect from light
Final solution (media)	DA neuron, cortical neuron, and astrocyte differentiation; midbrain organoid culture	1x	• Dilute stock solution 1:100	4°C	• Protect from light
	Motor neuron differentiation, cerebral organoid culture	0.5x	• Dilute stock solution 1:200	4°C	• Protect from light

β-Nerve growth factor (β-NGF)

Peprotech #450-01

- Neurotrophic factor that is crucial for development and preservation of sensory and sympathetic nervous systems

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	13.5 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	100 µg/mL		• Dissolve 500 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Cholinergic neuron differentiation	100 ng/mL	• Dilute stock solution 1:1000	4°C	

Neurobasal (NB) media

Life Technologies #21103-049

- Basal media for long-term maintenance and maturation of pure pre-natal and embryonic neuronal cell populations

	 MW/Concentration		 Preparation instructions	 Storage	 Important information
As supplied	1x		• Solution	4°C	
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation; cerebral organoid culture	1x	• Add supplements as per protocol	4°C	

Neurotrophin-3 (NT3)

Peprotech #450-03

- Neurotrophic factor that promotes the growth and survival of nerve and glial cells

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		13.6 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL	• Dissolve 50 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Sensory neuron and oligodendrocyte differentiation	10 ng/mL	• Dilute stock solution 1:1000	4°C	
	Forebrain organoid culture	20 ng/mL	• Dilute stock solution 1:500	4°C	

Noggin

Peprotech #120-10C

- Inhibits TGF-β ligands during developmental processes

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	46 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	200 µg/mL		• Dissolve 500 µg in 2.5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	NPC induction, midbrain organoid culture	200 ng/mL	• Dilute stock solution 1:1000	4°C	

Penicillin-Streptomycin

Wisent #450-200-EL

- Contains the antibiotics penicillin and streptomycin

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied/ stock solution	100X		• Solution	–20°C	
Final solution (media)	PBMC reprogramming, MEF culture, midbrain and cerebral organoid culture	1x	• Dilute stock solution 1:100	4°C	

Platelet-derived growth factor–AA (PDGF-AA)

Peprotech #100-13A

- Mitogen involved in a number of biological processes, including embryonic neuron development

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	28.5 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	10 µg/mL		• Dissolve 50 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Oligodendrocyte differentiation	10 ng/mL	• Dilute stock solution 1:1000	4°C	

Phosphate-buffered saline (PBS)

Wisent #311-010-CL

- Balanced salt solution used for a variety of cell culture applications, such as washing cells and preparing reagents

	 MW/Concentration	 Preparation instructions	 Storage	 Important information
As supplied	1x	• Solution	Room temperature	

2-Phospho-L-ascorbic acid (AA2P) trisodium salt

Sigma #49752

- Long-acting ascorbic acid derivative that stimulates collagen expression and formation

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	322.05 g/mol		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	50 mg/mL		• Dissolve 500 mg in 10 mL sterile ddH_2O • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming	50 µg/mL	• Dilute stock solution 1:1000	4°C	

Poly-L-ornithine (PO)

Sigma #P3655

- Synthetic amino acid polymer coating that acts as a charge modifier

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	30–70 kDa		• Powder	–20°C	
Stock solution	1 mg/mL		• Dissolve 100 mg in 100 mL sterile 1x PBS • Prepare 10-mL aliquots in 15-mL tubes	–20°C	
Final solution (coating)	DA neuron, cortical neuron, motor neuron, and astrocyte differentiation	10 µg/mL	• Dilute stock solution 1:100 in 1x PBS	4°C for up to 2 months	• Ensure transfer of all stock solution by rinsing tube with 1x PBS twice

Polyvinyl alcohol (PVA)

Sigma #P8136

- Hydrophilic linear polymer which forms copolymers of vinyl alcohol and vinyl acetate

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	28.5 kDa		• Powder	–20°C	
Stock solution	10 mg/mL		• Dissolve 100 mg in 10 mL ddH_2O • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (coating)	Microglia differentiation	10 µg/mL	• Dilute stock solution 1:1000	4°C	

Purmorphamine

Sigma #SML-0868

- Binds to the Smo receptor and activates Hedgehog signaling

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		520.6 g/mol	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		2 mM	• Dissolve 5 mg in 4.802 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Prepare as accurately as possible because the working concentration range is very narrow
Final solution (media)	DA neuron differentiation	2 µM	• Dilute stock solution 1:1000	4°C	• Use the smallest tip and a well-calibrated pipette
	Motor NPC induction (step 2 and 3 media)	0.5 µM	• Dilute stock solution 1:4000	4°C	
	Motor neuron differentiation	0.1 µM	• Dilute stock solution 1:20,000	4°C	

Culture Reagents: Q–Z

Retinoic acid (RA)

Sigma #R2625

- Activates transcription factors that regulate cell growth and differentiation

			MW/Concentration	Preparation instructions	Storage	Important information
As supplied			300.44 g/mol	• Powder; briefly centrifuge vial before opening	–20°C for up to 1 year	• Stable if unopened in ampule • Protect from light and air
Stock solution			1 mM	• Dissolve 300 mg in 10 mL DMSO • Dilute solution 1:10 in EtOH • Prepare 20-µL aliquots in 0.7-mL tubes	–80°C	• Use all of the powder immediately after opening the ampule • Protect from light and air
Final solution (media)	Motor neuron differentiation	0.5 µM		• Dilute stock solution 1:2000	4°C	• Always use a new aliquot of stock solution to prepare the final solution • Protect from light and air
	Motor NPC induction (step 2 and 3 media)	0.1 µM		• Dilute stock solution 1:10,000	4°C	

SB431542

Selleckchem #S1067

- Inhibits ALK5 and the TGF-β/Activin/NODAL pathway

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		384.4 g/mol	• Powder; briefly centrifuge vial before opening	–20°C for up to 3 years	
Stock solution		10 mM	• Dissolve 10 mg in 2.604 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C for up to 2 years	
Final solution (media)	DA NPC and cortical NPC induction, astrocyte differentiation*, midbrain organoid culture	10 µM	• Dilute stock solution 1:1000	4°C	
	Motor neuron and astrocyte differentiation*	2 µM	• Dilute stock solution 1:5000	4°C	

*Final solution concentration for astrocyte differentiation depends on protocol.

Smoothened Agonist (SAG)

Millipore #566660

- Activates Smo signalling, Smo internalization, and Hedgehog signaling

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	599 g/mol		• Powder; briefly centrifuge vial before opening	–20°C	• Can be stored at 4°C • Protect from light
Stock solution	1 mM		• Dissolve 1 mg in 1.669 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Can be stored at –20°C for up to 6 months • Protect from light
Final solution (media)	NPC induction	0.25 µM	• Dilute stock solution 1:4000	4°C	• Protect from light

Sodium butyrate

Sigma #B5887

- Inhibits histone deacetylases

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	110 g/mol		• Powder; briefly centrifuge vial before opening	Room temperature	
Stock solution	125 mM		• Dissolve 250 mg in 18.18 mL sterile ddH_2O • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming	250 µM	• Dilute stock solution 1:500	4°C	

Sonic hedgehog (SHH)

Peprotech #100-45

- Morphogen that activates Hedgehog signaling and is involved in central nervous system patterning during development

			MW/Concentration	Preparation instructions	Storage	Important information
As supplied			20.0 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution			200 µg/mL	• Dissolve 500 µg in 2.5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	NPC induction	200 ng/mL		• Dilute stock solution 1:1000	4°C	
	Midbrain organoid culture	100 ng/mL		• Dilute stock solution 1:2000	4°C	

Sonic hedgehog (SHH; C24II)

GenScript #Z03067

- Morphogen that activates Hedgehog signaling and is involved in central nervous system patterning during development

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	20.0 kDa	• Powder; briefly centrifuge vial before opening	–80°C for up to 6 months from date of receipt	
Stock solution	200 µg/mL	• Dissolve 500 µg in 2.5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Can be stored at 4°C for up to 2 weeks or at –20°C for up to 3 months
Final solution (media)	NPC induction 200 ng/mL	• Dilute stock solution 1:1000	4°C	

Stem cell factor (SCF)

Peprotech #300-07

- Hematopoietic growth factor that is essential for the survival, proliferation and differentiation of hematopoietic cells committed to the melanocyte and germ cell lineages

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		18.4 kDa	• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		10 µg/mL	• Dissolve 50 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	PBMC reprogramming	100 ng/mL	• Dilute stock solution 1:100	4°C	

Stemline II hematopoietic stem cell expansion medium

Sigma #S0192

- Hematopoietic stem cell expansion media for differentiated and undifferentiated cells

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	1x	• Solution	4°C	• Protect from light
Final solution (media)	1x	• Add supplements as per protocol	4°C	• Protect from light

StemPro Accutase Cell Dissociation Reagent

ThermoFisher #A1110501

- Cell detachment solution of proteolytic and collagenolytic enzymes

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	DA NPCs and cortical NPCs, midbrain organoids	1x	• Solution	• Upon arrival: –20°C • After thawing: 4°C for up to 2 years	• Multiple freeze-thaw cycles are not recommended • Do not store at room temperature • Protect from light

Thiazovivin

Tocris #3845

- Inhibits ROCK and the RHO/ROCK pathway

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	311 g/mol		• Powder; briefly centrifuge vial before opening	–20°C for up to 6 months	
Stock solution	2 mM		• Dissolve 10 mg in 16.367 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–20°C for up to 1 month	
Final solution (media)	iPSC culture; DA neuron, cortical neuron, and motor neuron differentiation	2 µM	• Dilute stock solution 1:1000	4°C	

1-Thioglycerol

Sigma #M1753

- Stimulates cell proliferation

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	1.25 g/mL (11.57 M)		• Solution	4°C	
Stock solution	0.4 M		• Dilute in sterile ddH_2O to achieve 0.4 M • Prepare 100-µL aliquots in 0.7-mL tubes	4°C	
Final solution (media)	PBMC reprogramming	0.4 mM	• Dilute stock solution 1:1000	4°C	

Thrombopoietin (TPO)

Peprotech #300-18

- Growth factor that stimulates the proliferation and maturation of megakaryocytes, and promotes increased circulating levels of platelets

		MW/Concentration		Preparation instructions	Storage	Important information
As supplied		18.4 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution		50 µg/mL		• Dissolve 250 µg in 5 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Microglia differentiation	50 ng/mL		• Dilute stock solution 1:1000	4°C	

Transforming growth factor β3 (TGF-β3)

Peprotech #100-36E

- Cytokine that regulates cell proliferation, growth, differentiation and motility, as well as synthesis and deposition of the extracellular matrix

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	25.0 kDa		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	1 µg/mL		• Dissolve 50 µg in 50 mL sterile ddH_2O containing 0.1% BSA • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron and cortical neuron differentiation	1 ng/mL	• Dilute stock solution 1:1000	4°C	

3,3′,5-Triiodo-L-thyronine (T3)

Sigma #T6397

- Thyroid hormone that regulates cell differentiation and protein expression

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	672.96 g/mol		• Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	60 µg/mL		• Dissolve 6 mg in 10 mL 0.1N NaOH • Add 1 mL of solution to 9 mL 1x PBS • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	• Can be stored at 4°C for up to 1 month or at –20°C • Avoid repeated freezing and thawing of aliquots
Final solution (media)	Oligodendrocyte differentiation	60 ng/mL	• Dilute stock solution 1:1000	4°C	

Valproic acid (VPA)

Sigma #P4543

- Anticonvulsant; inhibits histone deacetylates and enzymes involved in GABA metabolism

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		166.2 g/mol	• Powder	Room temperature	
Stock solution		0.5 M	• Dissolve 831 mg in 10 mL ddH_2O • Filter solution • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	0.5 mM	• Dilute stock solution 1:1000	4°C	
	Astrocyte differentiation	10 µM	• Dilute stock solution 1:50,000	4°C	

XAV939

Sigma #X3004

- Inhibits Tankyrase and Wnt/β-catenin signaling

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	312 g/mol		• Powder; briefly centrifuge vial before opening	–20°C	• Can be stored at room temperature
Stock solution	10 mM		• Dissolve 25 mg in 8.012 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	GABAergic neuron differentiation	1 µM	• Dilute stock solution 1:1000	4°C	

Y-27632

Selleckchem #S1049

- Inhibits ROCK

	MW/Concentration		Preparation instructions	Storage	Important information
As supplied	320.26 g/mol		• Powder; briefly centrifuge vial before opening	–20°C for up to 3 years	
Stock solution	10 mM		• Dissolve 50 mg in 15.612 mL sterile ddH_2O • Prepare 200-µL aliquots in 0.7-mL tubes	–80°C for up to 2 years	
Final solution (media)	iPSC culture; DA neuron, cortical neuron, and motor neuron differentiation; midbrain organoid culture	10 µM	• Dilute stock solution 1:1000	4°C	
	Cerebral organoid culture	50 µM	• Dilute stock solution 1:200	4°C	

You have reached the end of the protocol.