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iPSC Protocols

Culture Reagents

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v2.0



Antibiotic-Antimycotic

Gibco #15240-062

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



MW/Concentration

Preparation instructions

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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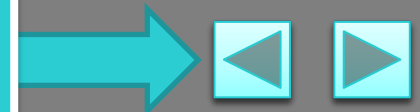


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Culture reagents: A–H

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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





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](#)
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- [Embryonic stem-cell fetal bovine serum \(FBS\)](#)
- [Epidermal growth factor \(EGF\)](#)
- [Erythropoietin \(EPO\)](#)
- [Essential 8 supplement](#)
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- [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](#)





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 \(\$\beta\$ -mercaptoethanol \[BME\]; Gibco #21985023\)](#)
- [2-mercaptoethanol \(\$\beta\$ -mercaptoethanol \[BME\]; Merck #8057400005\)](#)
- [Mitomycin C](#)
- [mTeSR1 5x supplement](#)
- [mTeSR1 basal media](#)
- [N-2 supplement](#)
- [\$\beta\$ -Nerve growth factor \(\$\beta\$ -NGF\)](#)
- [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- \$\beta\$ 3 \(TGF- \$\beta\$ 3\)](#)
- [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		<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C
Stock solution	12.5 µg/mL		<ul style="list-style-type: none">Dissolve 100 ug in 8 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Microglia differentiation	12.5 ng/mL	<ul style="list-style-type: none">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 ddH₂O
- Filter solution
- Prepare 200- μ L aliquots in 0.7-mL tubes

-80°C

Final solution (media)

DA neuron or cortical neuron differentiation

200 μ M

- Dilute stock solution 1:1000

4°C

Motor neuron differentiation, midbrain organoid culture

100 μ M

- 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

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied/ stock solution	50x	<ul style="list-style-type: none"> • Solution 	-20°C for 12 months from date of manufacture	<ul style="list-style-type: none"> • Protect from light
Final solution (media)	Forebrain and midbrain organoid culture	1x <ul style="list-style-type: none"> • Dilute stock solution 1:50 	4°C	<ul style="list-style-type: none"> • Protect from light
	Cerebral organoid culture	0.5x <ul style="list-style-type: none"> • Dilute stock solution 1:100 	4°C	<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none">• Powder	4°C
Stock solution	100 µg/mL		<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C	<ul style="list-style-type: none">Stable at 4°C but should be kept at -20°C for long-term storage
Stock solution		50 µg/mL	<ul style="list-style-type: none">Dissolve 100 mg in 2 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">Stable at 4°C for up to 1 weekAvoid repeated freeze-thaw cycles
Final solution (media)	Microglia differentiation	50 ng/mL	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C	



Bone morphogenetic protein 9 (BMP9)/GDF-2



Peprtech #120-07

- Regulates cell differentiation and survival



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	24.1 kDa		<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">Dissolve 200 µg in 20 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Cholinergic neuron differentiation	10 ng/mL	<ul style="list-style-type: none">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		<ul style="list-style-type: none"> • Powder; briefly centrifuge vial before opening 	-20°C
Stock solution	20 µg/mL		<ul style="list-style-type: none"> • Dissolve 500 µg in 25 mL sterile ddH₂O 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	<ul style="list-style-type: none"> • Dilute stock solution 1:1000 	4°C
	Motor neuron differentiation, midbrain organoid culture	10 ng/mL	<ul style="list-style-type: none"> • Dilute stock solution 1:2000 	4°C



CD200



Novoprotein #C311

- Immunoregulatory protein



MW/Concentration



Preparation instructions



Storage



Important information

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	30 kDa	<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C	<ul style="list-style-type: none">• Stable at room temperature for up to 3 weeks
Stock solution	100 µg/mL	<ul style="list-style-type: none">• Dissolve 200 µg in 2 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C for up to 3 months	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C	



CHIR-99021



Selleckchem #S2924

- Inhibits GSK-3 α/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- μ L 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 μ M

- Dilute stock solution 1:1000

4°C

Motor NPC induction (step 2 media)

1 μ M

- Dilute stock solution 1:3000

4°C

Midbrain organoid culture

0.8 μ M

- 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">• Dissolve 250 µg in 25 mL sterile ddH₂O 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	<ul style="list-style-type: none">• 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- μ L 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 μ M

- 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	100 µg/mL		<ul style="list-style-type: none">• Dissolve 500 µg in 5 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Microglia differentiation	100 ng/mL	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C for up to 3 years
Stock solution	10 mM		<ul style="list-style-type: none">• 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 μ M	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	4°C	
Stock solution	10 mM		<ul style="list-style-type: none">• Dissolve 25 mg in 6.37 mL DMSO• Prepare 20-μL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">• Avoid repeated freeze-thaw cycles
Final solution (media)	PBMC reprogramming	1 μ M	<ul style="list-style-type: none">• Dilute stock solution 1:10,000	4°C	



Dibutyryl-cAMP (db-cAMP)



Carbosynth #ND07996

- cAMP analog that activates cAMP-dependent kinases



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	491.37 g/mol	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C	
Stock solution	0.5 M	<ul style="list-style-type: none">Dissolve 1 g in 4.07 mL sterile ddH₂OPrepare 200-µL aliquots in 0.7-mL tubes	-80°C	
Final solution (media)	DA neuron and cortical neuron differentiation	0.5 mM	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C
	Midbrain organoid culture	125 µM	<ul style="list-style-type: none">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- μ L 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 μ M

- Dilute stock solution 1:10,000

4°C

Forebrain organoid culture

10 μ M

- 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- μ L aliquots in 0.7-mL tubes

-80°C
for up to 2 years

Final solution (media)

Motor neuron and astrocyte differentiation

2 μ M

- 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	<ul style="list-style-type: none">Solution	4°C for up to 12 months from date of manufacture	<ul style="list-style-type: none">Protect from light	
Final solution (coating)	Laminin solution	1x	<ul style="list-style-type: none">Add Antibiotic-Antimycotic and laminin as per protocol	4°C	<ul style="list-style-type: none">Protect from light
Final solution (media)	iPSC culture; DA neuron, cortical neuron, motor neuron, and astrocyte differentiation; cerebral organoid culture	1x	<ul style="list-style-type: none">Add supplements as per protocol	4°C	<ul style="list-style-type: none">Protect from light



DMEM



Wisent Bioproducts #319-005-CL

- Basal media for supporting growth of mammalian cells



MW/Concentration



Preparation instructions



Storage



Important instructions

	MW/Concentration	Preparation instructions	Storage	Important instructions
As supplied	1x	<ul style="list-style-type: none">Solution	4°C	
Final solution (media)	MEF culture	1x	<ul style="list-style-type: none">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)



Peprtech #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 ddH₂O 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)



Peprtech #100-64

- Hormone that stimulates proliferation and differentiation of erythroid progenitor cells



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	150 U/ μ g*		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	2000 U/ μ L		<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none"> • Solution 	<p>4°C for up to 12 months from date of manufacture</p>	<ul style="list-style-type: none"> • Protect from light
Final solution (media)	iPSC culture 1x	<ul style="list-style-type: none"> • 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 	<p>4°C for up to 2 weeks</p> <p>-20°C for up to 6 months</p>	<ul style="list-style-type: none"> • 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



Peprtech #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		<ul style="list-style-type: none"> Powder; briefly centrifuge vial before opening 	−20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none"> Dissolve 1 mg in 100 mL sterile ddH₂O 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	<ul style="list-style-type: none"> Dilute stock solution 1:1000 	4°C
	Astrocyte differentiation*	20 ng/mL	<ul style="list-style-type: none"> Dilute stock solution 1:500 	4°C
	Cerebral organoid culture	4 ng/mL	<ul style="list-style-type: none"> Dilute stock solution 1:2500 	4°C

*Final solution concentration for astrocyte differentiation depends on protocol.



Fibroblast growth factor 8 (FGF-8)



Peprtech #100-25

- Growth factor that promotes cell proliferation and differentiation



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	22.5 kDa		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	100 µg/mL		<ul style="list-style-type: none">• Dissolve 500 µg in 5 mL sterile ddH₂O 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	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	Room temperature	
Stock solution	100 mM		<ul style="list-style-type: none">• Dissolve 100 mg in 406 μL sterile ddH₂O• Prepare 20-μL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">• Stable at 4°C for up to 2 weeks• Protect from light
Final solution (media)	DA neuron, cortical neuron, and motor neuron differentiation	1 μ M	<ul style="list-style-type: none">• Dilute stock solution 1:100,000	4°C	<ul style="list-style-type: none">• 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

**As
supplied**

iPSCs, DA NPCs,
cortical NPCs, and
motor NPCs



Preparation instructions

1x • Solution



Storage

Room temperature



Important instructions



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 ddH₂O 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

	MW/Concentration	Preparation instructions	Storage
As supplied/ stock solution	100x (200 mM in 0.85% NaCl)	<ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 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

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	Mixture of polyanion chains of MW 6–30 kDa	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	–20°C	
Stock solution	20 mg/mL	<ul style="list-style-type: none">Dissolve 477 mg in 23.85 mL 1x PBSPrepare 50-µL aliquots in 0.7-mL tubes	–80°C	
Final solution (media)	Astrocyte differentiation	2 µg/mL	<ul style="list-style-type: none">Dilute stock solution 1:10,000	4°C
	Midbrain and cerebral organoid culture	1 µg/mL	<ul style="list-style-type: none">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 ddH₂O containing 0.1% BSA
- Prepare 200- μ L aliquots in 0.7-mL tubes

-80°C

Final solution (media)

Midbrain organoid culture

2.5 μ g/mL

- Dilute stock solution 1:2000

4°C

Cerebral organoid culture

1.25 μ g/mL

- Dilute stock solution 1:4000

4°C



Insulin-like growth factor 1 (IGF-1)



Peprtech #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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">• Dissolve 1 mg in 100 mL sterile ddH₂O 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	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">Dissolve 1 mg in 100 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	PBMC reprogramming	10 ng/mL	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C



Interleukin-6 (IL-6)



Peprtech #200-06

- Cytokine with diverse biological functions



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	20.9 kDa		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	50 µg/mL		<ul style="list-style-type: none">• Dissolve 500 µg in 10 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Microglia differentiation	50 ng/mL	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C



Interleukin-34 (IL-34)



Peprtech #200-34

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



MW/Concentration



Preparation instructions



Storage



Important information

As supplied		52.5 kDa	<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C	
Stock solution		100 µg/mL	<ul style="list-style-type: none">• Dissolve 500 µg in 5 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C	
Final solution (media)	Microglia differentiation	100 ng/mL	<ul style="list-style-type: none">• 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

		MW/Concentration	Preparation instructions	Storage	Important information
As supplied		1x	<ul style="list-style-type: none"> • Solution 	4°C	
Final solution (media)	PBMC reprogramming, iPSC culture	1x	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	32 mg/mL		<ul style="list-style-type: none">• Dissolve 32 mg in 1 mL sterile ddH₂O• Prepare 100-µL aliquots in 0.7-mL tubes	4°C
Final solution (media)	PBMC reprogramming	64 µg/mL	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	1 mM	<ul style="list-style-type: none">• Dissolve 5 mg in 12.3 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Astrocyte differentiation 0.1 µM	<ul style="list-style-type: none">• 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

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	8 M in H ₂ O	<ul style="list-style-type: none">• Solution	4°C	
Stock solution	2 M	<ul style="list-style-type: none">• Dilute in sterile ddH₂O to achieve 2 M• Filter solution• Prepare 100-µL aliquots in 0.7-mL tubes	4°C	
Final solution (media)	PBMC reprogramming 2 mM	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C	



Macrophage colony stimulating factor (M-CSF)



Peprtech #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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	25 µg/mL		<ul style="list-style-type: none">• Dissolve 500 µg in 20 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Microglia differentiation	25 ng/mL	<ul style="list-style-type: none">• 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 ddH₂O (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 μ M) 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	<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	4°C		
Stock solution	1 mg/mL	<ul style="list-style-type: none">• Dissolve 2 mg in 2 mL sterile ddH₂O• Prepare 50-µL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none"> • Solution 	<p>–20°C for up to 3 months</p>	
Final solution (media)	iPSC culture 1x	<ul style="list-style-type: none"> • 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 	<p>4°C for up to 2 weeks</p> <p>–20°C for up to 6 months</p>	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Solution 	4°C	
Final solution (media)	iPSC culture 1x	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 μ g/mL

- Dissolve 500 μ g in 5 mL sterile ddH₂O containing 0.1% BSA
- Prepare 100- μ L 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)



Peprtech #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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">• Dissolve 50 µg in 5 mL sterile ddH₂O 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	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C
	Forebrain organoid culture	20 ng/mL	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	200 μ g/mL		<ul style="list-style-type: none">• Dissolve 500 μg in 2.5 mL sterile ddH₂O 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	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">• Dissolve 50 µg in 5 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Oligodendrocyte differentiation	10 ng/mL	<ul style="list-style-type: none">• 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

As supplied

1x



Preparation instructions

- Solution



Storage

Room temperature



Important information



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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	50 mg/mL		<ul style="list-style-type: none">• Dissolve 500 mg in 10 mL sterile ddH₂O• Filter solution• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	PBMC reprogramming	50 µg/mL	<ul style="list-style-type: none">• 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

	MW/Concentration	Preparation instructions	Storage	Important information
As supplied	30–70 kDa	<ul style="list-style-type: none">• Powder	–20°C	
Stock solution	1 mg/mL	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• Dilute stock solution 1:100 in 1x PBS	4°C for up to 2 months	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">Powder	-20°C
Stock solution	10 mg/mL	<ul style="list-style-type: none">Dissolve 100 mg in 10 mL ddH₂OFilter solutionPrepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (coating)	Microglia differentiation 10 µg/mL	<ul style="list-style-type: none">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

	MW/Concentration	Preparation instructions	Storage	Important information	
As supplied	520.6 g/mol	<ul style="list-style-type: none"> • Powder; briefly centrifuge vial before opening 	-20°C		
Stock solution	2 mM	<ul style="list-style-type: none"> • Dissolve 5 mg in 4.802 mL DMSO • Prepare 100-µL aliquots in 0.7-mL tubes 	-80°C	<ul style="list-style-type: none"> • Prepare as accurately as possible because the working concentration range is very narrow 	
Final solution (media)	DA neuron differentiation	2 µM	<ul style="list-style-type: none"> • Dilute stock solution 1:1000 	4°C	
	Motor NPC induction (step 2 and 3 media)	0.5 µM	<ul style="list-style-type: none"> • Dilute stock solution 1:4000 	4°C	<ul style="list-style-type: none"> • Use the smallest tip and a well-calibrated pipette
	Motor neuron differentiation	0.1 µM	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Powder; briefly centrifuge vial before opening 	-20°C for up to 1 year	<ul style="list-style-type: none"> • Stable if unopened in ampule • Protect from light and air
Stock solution		1 mM	<ul style="list-style-type: none"> • Dissolve 300 mg in 10 mL DMSO • Dilute solution 1:10 in EtOH • Prepare 20-µL aliquots in 0.7-mL tubes 	-80°C	<ul style="list-style-type: none"> • Use all of the powder immediately after opening the ampule • Protect from light and air
Final solution (media)	Motor neuron differentiation	0.5 µM	<ul style="list-style-type: none"> • Dilute stock solution 1:2000 	4°C	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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- μ L 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 μ M

- Dilute stock solution 1:1000

4°C

Motor neuron and astrocyte differentiation*

2 μ M

- 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	<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C	<ul style="list-style-type: none">• Can be stored at 4°C• Protect from light
Stock solution		1 mM	<ul style="list-style-type: none">• Dissolve 1 mg in 1.669 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-μL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">• Can be stored at -20°C for up to 6 months• Protect from light
Final solution (media)	NPC induction	0.25 μM	<ul style="list-style-type: none">• Dilute stock solution 1:4000	4°C	<ul style="list-style-type: none">• Protect from light



Sodium butyrate



Sigma #B5887

- Inhibits histone deacetylases



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	110 g/mol		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	Room temperature
Stock solution	125 mM		<ul style="list-style-type: none">• Dissolve 250 mg in 18.18 mL sterile ddH₂O• Prepare 100-μL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	PBMC reprogramming	250 μ M	<ul style="list-style-type: none">• Dilute stock solution 1:500	4°C



Sonic hedgehog (SHH)



Peprtech #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	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C
Stock solution		200 µg/mL	<ul style="list-style-type: none">Dissolve 500 µg in 2.5 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	NPC induction	200 ng/mL	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C
	Midbrain organoid culture	100 ng/mL	<ul style="list-style-type: none">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	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-80°C for up to 6 months from date of receipt	
Stock solution	200 µg/mL	<ul style="list-style-type: none">Dissolve 500 µg in 2.5 mL sterile ddH₂O containing 0.1% BSAPrepare 100-µL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">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	<ul style="list-style-type: none">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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	10 µg/mL		<ul style="list-style-type: none">• Dissolve 50 µg in 5 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	PBMC reprogramming	100 ng/mL	<ul style="list-style-type: none">• 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

As supplied

DA NPCs and cortical NPCs, midbrain organoids

1x



Preparation instructions

- Solution



Storage

- Upon arrival: -20°C
- After thawing: 4°C for up to 2 years



Important information

- 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)		<ul style="list-style-type: none">Solution	4°C
Stock solution	0.4 M		<ul style="list-style-type: none">Dilute in sterile ddH₂O to achieve 0.4 MPrepare 100-µL aliquots in 0.7-mL tubes	4°C
Final solution (media)	PBMC reprogramming	0.4 mM	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C



Thrombopoietin (TPO)



Peprtech #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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	50 µg/mL		<ul style="list-style-type: none">• Dissolve 250 µg in 5 mL sterile ddH₂O containing 0.1% BSA• Prepare 100-µL aliquots in 0.7-mL tubes	-80°C
Final solution (media)	Microglia differentiation	50 ng/mL	<ul style="list-style-type: none">• Dilute stock solution 1:1000	4°C



Transforming growth factor $\beta 3$ (TGF- $\beta 3$)



Peprtech #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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C
Stock solution	1 μ g/mL		<ul style="list-style-type: none">• Dissolve 50 μg in 50 mL sterile ddH₂O 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	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">Powder; briefly centrifuge vial before opening	-20°C	
Stock solution	60 µg/mL	<ul style="list-style-type: none">Dissolve 6 mg in 10 mL 0.1N NaOHAdd 1 mL of solution to 9 mL 1x PBSFilter solutionPrepare 100-µL aliquots in 0.7-mL tubes	-80°C	<ul style="list-style-type: none">Can be stored at 4°C for up to 1 month or at -20°CAvoid repeated freezing and thawing of aliquots
Final solution (media)	Oligodendrocyte differentiation 60 ng/mL	<ul style="list-style-type: none">Dilute stock solution 1:1000	4°C	



Valproic acid (VPA)



Sigma #P4543

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



MW/Concentration



Preparation instructions



Storage



Important information

As supplied	166.2 g/mol	<ul style="list-style-type: none">• Powder	Room temperature
Stock solution	0.5 M	<ul style="list-style-type: none">• Dissolve 831 mg in 10 mL ddH₂O• 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	<ul style="list-style-type: none">• Dilute stock solution 1:1000 4°C
	Astrocyte differentiation	10 μ M	<ul style="list-style-type: none">• 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		<ul style="list-style-type: none">• Powder; briefly centrifuge vial before opening	-20°C	<ul style="list-style-type: none">• Can be stored at room temperature
Stock solution	10 mM		<ul style="list-style-type: none">• 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 μ M	<ul style="list-style-type: none">• 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 ddH₂O
- 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.

