

Materials List for:

Generation of Human Primordial Germ Cell-like Cells at the Surface of Embryoid Bodies from Primed-pluripotency Induced Pluripotent Stem Cells

Shino Mitsunaga¹, Keiko Shioda¹, Kurt J. Isselbacher¹, Jacob H. Hanna², Toshi Shioda¹

¹Center for Cancer Research, Massachusetts General Hospital

²Department of Molecular Genetics, Weizmann Institute of Science

Correspondence to: Toshi Shioda at TSHIODA@mgh.harvard.edu

URL: <https://www.jove.com/video/58297>

DOI: [doi:10.3791/58297](https://doi.org/10.3791/58297)

Materials

Name	Company	Catalog Number	Comments
Primed pluripotency hiPSC culture			
Matrigel	Corning	354277	Aliquot Matrigel at the volume indicated by the manufacturer (about 200 µL). Use cold tubes. Store at -80 °C
DMEM/F-12	Thermo Fisher Scientific	21041-025	Store at 4 °C.
mTeSR1	STEMCELL Technologies	85850	Reconstitute by adding 5X Supplement to Basal Medium. The reconstituted medium can be stored at 4 °C for up to 4 weeks without affecting cell culture performance.
Y27632	Axon	1683	Dilute 5 mg Y27632 (MW 320.26) with 312.2 µL water to prepare 50 mM Y27632 stock solution. Sterilize by filtration (0.22 µm). Aliquot 20 µL/tube x 15 tubes and store at -20 °C.
CryoStor CS10	STEMCELL Technologies	07930	Store at 4 °C.
Accutase	Innovative cell technologies	AT104-500	Cell dissociation enzyme; aliquot 40 mL/tube x 12 tubes and store at -20 °C.
Name	Company	Catalog Number	Comments
4i hiPSC culture			
KnockOut DMEM	Thermo Fisher Scientific	A1286101	
KnockOut Serum Replacement	Thermo Fisher Scientific	10828028	Aliquot 40 mL/tube x 12 tubes and store at -20 °C.
Penicillin-Streptomycin-Glutamine (100x)	Thermo Fisher Scientific	10378016	
MEM Non-Essential Amino Acids Solution (100x)	Thermo Fisher Scientific	11140050	
Insulin from bovine pancreas	Sigma-Aldrich	I1882-100MG	Add 0.1 mL glacial acetic acid to 10 mL water. Sterilize by filtration (0.22 µm). Dilute 100 mg insulin lyophilized powder with cold 10 mL the acidified water to make 10 mg/mL stock solution. Aliquot 650 µL/tube x 15 tubes and store at -80 °C.
human LIF	PeproTech	300-05	Reconstitute 250 µg human LIF with 250 µL water. Add 750 µL of 0.1% bovine serum albumin in

			PBS(-) to make 250 µg/mL human LIF stock solution. Specific activity of this product is >10,000 units/µg. Aliquot 40 µL/tube x 25 tubes and store at -80 °C.
human FGF2	R&D Systems	4114-TC	Reconstitute 1 mg human FGF2 with 5 mL PBS(-) to make 200 µg/mL human FGF2 stock solution. Aliquot 100 µL/tube x 50 tubes and store at -80 °C.
human TGF-β1	PeptoTech	100-21	Reconstitute 50 µg with 50 µL of 10 mM Citric Acid (pH 3.0). Add 150 µL of 0.1% bovine serum albumin in PBS(-) to make 250 µg/mL human TGF-β1 stock solution. Aliquot 4 µL/tube x 50 tubes and store at -80 °C.
4i basal medium			Mix the following reagents to make 4i basal medium. 500 mL of KnockOut DMEM 100 mL of KnockOut Serum Replacement 6 mL of Penicillin-Streptomycin-Glutamine (100x) 6 mL of MEM Non-Essential Amino Acids Solution (100x) 650 µL of 10 mg/mL Insulin from bovine pancreas 40 µL of 250 µg/mL human LIF 20 µL of 200 µg/ml human FGF2 4 µL of 250 µg/ml human TGF-β1 Aliquots 40 mL/tube and store at -80 °C.
CHIR99021	Axon	1386	Reconstitute 25 mg CHIR99021 (MW 465.34) with 1791 µL DMSO to make 30 mM CHIR99021 stock solution. Aliquot 50 µL/tube x 35 tubes and store at -20 °C.
PD0325901	Axon	1408	Reconstitute 5 mg PD0325901 (MW 482.19) with 1037 µL DMSO to make 10 mM PD0325901 stock solution. Aliquot 50 µL/tube x 20 tubes and store at -20 °C.
BIRB796	Axon	1358	Reconstitute 10 mg BIRB796 (MW 527.66) with 948 µL DMSO to make 20 mM BIRB796 stock solution. Aliquot 50 µL/tube x 18 tubes and store at -20 °C.
SP600125	Tocris	1496	Reconstitute 10 mg SP600125 (MW220.23) with 908 µL DMSO to make 50 mM SP600125 stock solution. Aliquot 50 µL/tube x 18 tubes and store at -20 °C.
AggreWell400	STEMCELL Technologies	27845	Microwell plate
Pluronic F-127	Sigma-Aldrich	P2443	Reconstitute 5 g Pluronic F-127 in 100 mL water to make 5%(w/v) Pluronic F127 stock solution. Sterilize by filtration (0.22 µm). Aliquot 50 mL/tube x 2 tubes and store at r.t.
Name	Company	Catalog Number	Comments
hPGCLC culture			
Glasgow's MEM	Thermo Fisher Scientific	11710035	

Sodium pyruvate (100 mM)	Thermo Fisher Scientific	11360070	
Penicillin-Streptomycin (100x)	Corning	30-002-CI	
hPGCLC basal medium			Mix the following reagents to make hPGCLC basal medium. 500 mL of Glasgow's MEM 75 mL of KnockOut Serum Replacement 6 mL of MEM Non-Essential Amino Acids Solution (100x) 6 mL of 100 mM Sodium pyruvate 6 mL of Penicillin-Streptomycin (x100) Aliquots 40 mL/tube and store at -20 °C.
2-Mercaptoethanol	Sigma-Aldrich	M3148	Dilute 350 µL 2-Mercaptoethanol (14.3 M) in 9.65 mL PBS(-) to make 500 mM 2-Mercaptoethanol stock solution . Store at 4 °C.
L-Ascorbic acid	Sigma-Aldrich	A4544	Reconstitute 400 mg L-Ascorbic acid with 20 mL water to make 20 mg/mL L-Ascorbic acid stock solution. Sterilize by filtration (0.22 µm). Aliquot 500 µL/tube x 40 tubes and store at -20 °C.
Recombinant human BMP4	R&D Systems	314-BP	Reconstitute 1 mg recombinant human BMP4 with 10 mL of 4mM HCl aq. to make 100 µg/mL recombinant human BMP4 stock solution. Aliquot 250 µL/tube x 40 tubes and store at -80 °C.
Human SCF	PeproTech	300-07	Reconstitute 250 µg human SCF with 500 µL of 0.1% bovine serum albumin in PBS(-) to make 500 µg/mL human SCF stock solution. Aliquot 35 µL/tube x 14 tubes and store at -80 °C.
Human EGF	PeproTech	AF-100-15	Reconstitute 1 mg human EGF with 1 mL of 0.1% bovine serum albumin in PBS(-) to make 1 mg/mL human EGF stock solution. Aliquot 100 µL/tube x 10 tubes. Dilute 100 µL of 1 mg/mL human EGF stock solution with 300 µL of 0.1% bovine serum albumin in PBS(-) to make 250 µg/mL humanEGF stock solution. Aliquot 20 µL/tube x 20 tubes. Store at -80 °C.
Cell strainer	Corning	352340	Pore size = 40 µm.
50 ml polypropylene conical tube	Corning	352070	
Low attachment plate	Corning	3471	
Vari-Mix Platform Rocker	Thermofisher	M79735Q	
Name	Company	Catalog Number	Comments
Immunohistochemical staining			
BLOXALL Blocking Solution	VECTOR	SP-6000	
Normal Horse serum ImmPRESS Reagent Anti-Goat IgG	VECTOR	MP-7405	
OCT-3/4 Antibody	Santa Cruz	SC-8629	
ImmPACT DAB	VECTOR	SK-4105	

Permout	Thermofisher	SP15-100	Mounting medium
Name	Company	Catalog Number	Comments
Isolating hPGCLC			
Embryoid Body Dissociation Kit	Miltenyi Biotec	130-096-348	
Anti-CD38 antibody conjugated to APC	abcam	ab134399	