

Generation of Patient-Derived Podocytes from Skin Biopsies

 Victoria Rose¹, Janina Müller-Deile^{1,2}
¹Department of Nephrology and Hypertension, University Hospital Erlangen, Friedrich-Alexander University (FAU) Erlangen-Nürnberg ²Research Center on Rare Kidney Diseases (RECORD), University Hospital Erlangen

Corresponding Authors

Victoria Rose

victoria.rose@uk-erlangen.de

Janina Müller-Deile

Janina.Mueller-Deile@uk-erlangen.de

Citation

 Rose, V., Müller-Deile, J. Generation of Patient-Derived Podocytes from Skin Biopsies. *J. Vis. Exp.* (195), e65364, doi:10.3791/65364 (2023).

Date Published

May 26, 2023

DOI

10.3791/65364

URL

jove.com/video/65364

Materials

Name	Company	Catalog Number	Comments
0.2 µm sterile filter	Rotilab	P668.1	for sterilization of differentiation medium
all-trans retinoic acid	Stem Cell Technologies	72262	supplement for differentiation
B27 supplement (50 x), serum free	Gibco	17504044	supplement for serum-free differentiation medium
BG iMatrix-511 Silk	biogems	RL511S	additional option of extracellular matrix reagent used in coating solution to coat cell culture plastics suitable for hiPSC culture
Bovine serum albumin (BSA)	Roth	8076.4	
CELLSTAR Filter Cap Cell Culture Flasks, T75, 250 mL	Greiner bio-one	82050-856	cell culture plastics suitable for fibroblast culture
CHIR99021 (5 mg)	Sigma-Aldrich	252917-06-9	supplement for differentiation
Corning Matrigel hESC qualified matrix	Corning	354277	additional option of extracellular matrix reagent used in coating solution to coat cell culture plastics suitable for hiPSC culture (solubilized basement membrane matrix)
countess Cell Counting Chamber Slides	Invitrogen	C10283	to count cells
countess II FL Automated Cell Counter	Invitrogen		to count cells
cryoPure tubes, 2 ml, QuickSeal screw cap, white	Sarstedt	72380	cryovials for freezing of cells
dimethyl sulfoxide (DMSO)	Roth	A994.1	for fibroblast freezing medium
DMEM/F12 (1:1) (1 x)	Gibco	11320074	basic medium for differentiation
DMEM/F12 + Glutamax	Gibco	10565018	basic medium for fibroblast medium
EVOS M5000 Imaging System	Thermo Fisher Scientific	AMF5000	phase contrast microscope
fetal bovine serum premium, inactivated (FCS)	PAN Biotech	P301902	serum for fibroblast medium, fibroblast freezing medium and podocyte maintenance medium

fisherbrand Electroporation Cuvettes Plus, 4 mm gap, 800 µL capacity, sterile	Fisherbrand	FB104	cuvette used for electroporation/ episomal reprogramming of fibroblasts (4mm gap)
fluoromount-G Mounting Medium, with DAPI	Invitrogen	00-4959-52	mounting medium containing dapi
gauge needle (0.6 x 30 mm)	BD Microlance3	300700	for separation of hiPSC colonies into small pieces
human Recombinant Activin A Protein	78001.1	Stem cell technologies	supplement for differentiation
human recombinant bone morphogenetic protein 7 (BMP7)	Peptrotech	120-03P	supplement for differentiation
human VEGF-165 Recombinant Protein	Thermo Scientific	PHC9394	supplement for differentiation
insulin-transferrin-selenium (ITS -G) (100 x)	Gibco	41400045	supplement for podocyte maintenance medium
LB medium	Roth	X964.1	for sterility test of hiPSC culture
lookOut Mycoplasma PCR Detection Kit	Sigma Aldrich	MP0035-1KT	commercial mycoplasma detection kit
microscope slides	Diagonal GmbH & Co.KG	21,102	
microtube 1.5 mL	Sarstedt	72706400	
mTeSR1 Complete Kit	Stem Cell Technologies	85850	basic medium for serum-free hiPSC culture medium
nalgene freezing container Mr.Frosty	Roth	AC96.1	to ensure optimal freezing conditions
normal goat serum	abcam	ab 7481	for preincubation solution and antibody diluent
nunc 24 well plates	Thermo Scientific	142485	cell culture plastics suitable for hiPSC culture
nunc 48 well plates	Thermo Scientific	152640	cell culture plastics suitable for hiPSC culture
nunc 6 well plates	Thermo Scientific	140685	cell culture plastics suitable for hiPSC culture
nunc EasYDish Dishes 100 mm	Thermo Scientific	150466	cell culture plastics suitable for hiPSC culture
nunc MicroWell 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate	Thermo Scientific	167008	cell culture plastics suitable for hiPSC culture
nutriFreez D10 Cryopreservation Medium	Sartorius	05-713-1E	serum-free cryopreservation medium for cryopreservation of hiPSC and nephron progenitor cells
Opti-MEM	Gibco	11058021	electroporation medium
pCXLE-hMLN	Addgene	#27079	plasmid for episomal reprogramming
pCXLE-hOCT3/4 plasmid	Addgene	#27077	plasmid for episomal reprogramming
pCXLE-hSK plasmid	Addgene	#27078	plasmid for episomal reprogramming
penicillin-streptomycin	Sigma-Aldrich	P4333-100ML	to avoid bacterial contamination
plastic coverslips	Sarstedt	83.1840.002	for immunofluorescent stainings of hiPSCs and hiPSC-derived podocytes
ROTI Histofix	Roth	P087.3	commercial paraformaldehyde (4 %) for fixation of cells
RPMI 1640 + L-Glutamine	Gibco	21875034	basic medium for podocyte maintenance medium
staining chamber StainTray Black lid	Roth	HA51.1	
stemPro Accutase Cell Dissociation Reagent	Gibco	A1110501	enzymatic cell detachment solution used for dissociation of hiPSCs
sterile phosphate buffered saline (PBS) (1 x)	Gibco	14190094	used for washing and coating

sterile water	Roth	T1432	
syringe without needle 20 mL	BD Plastipak	300629	to filter sterilize differentiation medium
TC dish 100 mm	Sarstedt	8,33,902	sterile cell culture plastics used for cutting the skin biopsy and fibroblast culture
TC dish 35 mm	Sarstedt	8,33,900	sterile cell culture plastics used for outgrowing fibroblasts from skin biopsy
triton X 100	Roth	3051.3	for preincubation solution
trypan Blue Stain (0.4 %) for use with the Countess Automated Cell Counter	Invitrogen	T10282	to count cells
trypsin-EDTA (10 x)	Biowest	X0930-100	dissociation reagent used for fibroblasts and nephron progenitor cells
tube 15 mL	Greiner bio-one	188271-N	
tube 50 mL	Greiner bio-one	227261	
vitronectin ACF	Sartorius	05-754-0002	extracellular matrix reagent used in coating solution to coat cell culture plastics suitable for hiPSC culture
Y-27632 dihydrochloride (10 mg)	Tocris	1254	to avoid apoptosis of hiPSCs during splitting
Primary antibodies			
OCT4	Stem Cell Technologies	60093.1	pluripotency marker, dilution 1:200
SSEA-4	Stem Cell Technologies	60062FI.1	pluripotency marker, dilution 1:100
Ki67	Abcam	ab15580	proliferation marker, dilution 1:300
synaptopodin	Proteintech	21064-1-AP	podocyte-specific marker, dilution 1:200
nephrin	Progen	GP-N2	podocyte-specific marker, dilution 1:25
podocin	proteintech	20384-1-AP	podocyte-specific marker, dilution 1:100
Secondary antibodies			
goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Invitrogen	A21435	secondary antibody, dilution 1:1000
alexa Fluor 647 Goat Anti-Rabbit SFX Kit, highly cross-adsorbed	Invitrogen	A31634	secondary antibody, dilution 1:1000
donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A21206	secondary antibody, dilution 1:1000
goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Invitrogen	A21422	secondary antibody, dilution 1:1000
goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A11001	secondary antibody, dilution 1:1000