

Guided Differentiation of Mature Kidney Podocytes from Human Induced Pluripotent Stem Cells Under Chemically Defined Conditions

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jove.com/video/61299

Materials

Name	Company	Catalog Number	Comments
Cells			
DU11 human iPS cells			The DU11(Duke University clone #11) iPS cell line was generated at the Duke University iPSC Core Facility and provided to us by the Bursac Lab at Duke University. This line has been tested for mycoplasma and was last karyotyped in July 2019 by our lab, and found to be karyotypically normal.
Growth Factors and Media Supplements			
All-trans retinoic acid (500 mg)	72262	Stem Cell Technologies	
B27 serum-free supplement	17504044	Thermo/Life Technologies	
CHIR99021	04-0004	Stemgent	May show lot-to-lot variation
Complete Medium Kit with CultureBoost-R	4Z0-500-R	Cell Systems	Podocyte maintenance media
DMEM/F12	12634028	Thermo/Life Technologies	
DMEM/F12 with GlutaMAX supplement	10565042	Thermo/Life Technologies	DMEM/F12 with glutamine supplement
Heat-inactivated FBS	10082147	Thermo/Life Technologies	
Human activin A	PHC9564	Thermo/Life Technologies	
Human BMP7	PHC9544	Thermo/Life Technologies	
Human VEGF	PHC9394	Thermo/Life Technologies	
mTeSR1 medium	05850	Stem Cell Technologies	hiPS cell culture media (CCM)
Penicillin–streptomycin, liquid (100×)	15140-163	Thermo/Life Technologies	
Y27632 ROCK inhibitor	1254	Tocris	
Antibodies			

Alexa Fluor 488– and Alexa Fluor 594–conjugated secondary antibodies	A32744; A32754; A-11076; A32790	Thermo/Life Technologies	
Brachyury(T)	ab20680	Abcam	
Nephrin	GP-N2	Progen	
OCT4	AF1759	R&D Systems	
PAX2	71-6000	Invitrogen	
WT1	MAB4234	Millipore	
ECM Molecules			
iMatrix-511 Laminin-E8 (LM-E8) fragment	N-892012	Iwai North America	Basement membrane (BM) matrix 2
Matrigel hESC-qualified matrix, 5-mL vial	354277	BD Biosciences	Basement membrane (BM) matrix 1. May show lot-to-lot variation
Enzymes and Other Reagents			
Accutase	A1110501	Thermo/Life Technologies	Cell detachment solution
BSA	A9418	Sigma-Aldrich	
Dimethyl Sulfoxide (DMSO)	D2438	Sigma-Aldrich	DMSO is toxic. Should be handled in chemical safety hood
Enzyme-free cell dissociation buffer, Hank's balanced salt	13150016	Thermo/Life Technologies	
Ethanol solution, 70% (vol/vol), biotechnology grade	97065-058	VWR	Ethanol is flammable and toxic
FBS	431097	Corning	
Paraformaldehyde (PFA)	28906	Thermo/Life Technologies	PFA should be handled in a chemical fume hood with proper personal protection equipment, including gloves, lab coat, and safety eye glasses. Avoid inhalation and contact with skin.
Phosphate-buffered saline (PBS)	14190-250	Thermo/Life Technologies	
Sterile Distilled Water	15230162	Thermo/Life Technologies	
Triton X-100	97062-208	VWR	
Trypsin-EDTA, 0.05%	25300-120	Thermo/Life Technologies	
Equipment			
Aspirating pipettes, individually wrapped	29442-462	Corning	
Avanti J-15R Centrifuge	B99516	Beckman Coulter	
Conical centrifuge tube, 15 mL	352097	Corning	
Conical centrifuge tube, 50 mL	352098	Corning	
Cryoboxes	3395465	Thermo/Life Technologies	For storing frozen aliquots
EVOS M7000	AMF7000	Thermo/Life Technologies	Flourescent microscope used to acquire images of fixed and stained iPS cells and their derivatives
Hemocytometer	100503-092	VWR	
Heracell VIOS 160i CO2 incubator	51030403	Thermo/Life Technologies	For the routine culture and maintenace of iPS cells and their derivatives
Inverted Zeiss Axio Observer equipped with AxioCam 503 camera	491916-0001-000(microscope) ; 426558-0000-000(camera)	Carl Zeiss Microscopy	Used to acquire phase contrast images of live iPS cells and their derivatives at each stage of podocyte differentiation
Kimberly-Clark nitrile gloves	40101-346	VWR	
Kimwipes, large	21905-049	VWR	
Kimwipes, small	21905-026	VWR	

P10 precision barrier pipette tips	P1096-FR	Denville Scientific	
P100 barrier pipette tips	P1125	Denville Scientific	
P1000 barrier pipette tips	P1126	Denville Scientific	
P20 barrier pipette tips	P1121	Denville Scientific	
P200 barrier pipette tips	P1122	Denville Scientific	
Serological pipette, 10 mL, individually wrapped	356551	Corning	
Serological pipette, 25 mL, individually wrapped	356525	Corning	
Serological pipette, 5 mL, individually wrapped	356543	Corning	
Steriflip, 0.22 µm, PES	SCGP00525	EMD Millipore	
Sterile Microcentrifuge Tubes	1138W14	Thomas Scientific	For aliquoting growth factors
Tissue culture–treated 12-well plates	353043	Corning	
Tissue culture–treated six-well plates	353046	Corning	
VWR white techuni lab coat	10141-342	VWR	
Wide-beveled cell lifter	3008	Corning	