

Materials List for:

# Reprogramming Primary Amniotic Fluid and Membrane Cells to Pluripotency in Xeno-free Conditions

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URL: <https://www.jove.com/video/56003>

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## Materials

Name	Company	Catalog Number	Comments
Tumor Dissociation Kit, human	Miltenyi Biotec	130-095-929	tissue dissociation system, reagent kit, includes tissue dissociation tubes and tissue dissociation enzymes
gentleMACS Dissociator	Miltenyi Biotec	130-093-235	tissue dissociation system, dissociator
Thermo Scientific™ Shandon™ Disposable Scalpel No. 10, Sterile, Individually Wrapped, 5.75 (14.6cm)	Thermo-Fisher	3120032	
70 µm cell strainers	Corning	10054-456	
RPMI 1640 medium	Thermo-Fisher	32404014	
rocking platform	VWR	40000-300	
50 ml centrifuge tubes	Thermo-Fisher	339652	
15 ml centrifuge tubes	Thermo-Fisher	339650	
EBM-2 basal medium	Lonza	CC-3156	basal medium for AFMC medium
FGF 2 Human (expressed in E. coli, non-glycosylated)	Prospec Bio	CYT-218	bFGF, supplement for AFMC medium
EGF Human, Pichia	Prospec Bio	CYT-332	EGF, supplement for AFMC medium
LR3 Insulin Like Growth Factor-1 Human Recombinant	Prospec Bio	CYT-022	IGF, supplement for AFMC medium
Fetal Bovine Serum, embryonic stem cell-qualified	Thermo-Fisher	10439024	FBS
Antibiotic-Antimycotic (100X)	Thermo-Fisher	15240062	for primary AFSC/AMSC, for routine AFSC/AMSC it should not be necessary, do not use in medium for transfected cells!
Accutase cell detachment solution	StemCell Technologies	07920	cell detachment enzyme
CryoStor™ CS10	StemCell Technologies	07930	complete freezing medium
PBS, pH 7.4	Thermo-Fisher Scientific	10010023	
EndoFree Plasmid Maxi Kit (10)	Qiagen	12362	for plasmid isolation
pEP4 E02S EN2K	Addgene	20925	EN2K, reprogramming factors Oct4+Sox2, Nanog+Klf4
pEP4 E02S ET2K	Addgene	20927	ET2K, reprogramming factors Oct4+Sox2, SV40LT+Klf4

pCEP4-M2L	Addgene	20926	M2L, reprogramming factors c-Myc +LIN28
NanoDrop 2000c UV-Vis Spectrophotometer	Thermo-Fisher	ND-2000C	spectrophotometer
Neon® Transfection System	Thermo-Fisher	MPK5000	transfection system, components: Neon pipette - transfection pipette Neon device - transfection device
Neon® Transfection System 10 µL Kit	Thermo-Fisher	MPK1025	consumables kit for the Neon Transfection System, it contains: Neon tip - transfection tip Neon tube - transfection tube buffer R - resuspension buffer buffer E - electrolytic buffer
Stemolecule™ Sodium Butyrate	StemGent	04-0005	small molecule enhancer of reprogramming
TeSR-E8	StemCell Technologies	05940	E8 medium
Vitronectin XF™	StemCell Technologies	07180	VTN, stock concentration 250 µg/ml, used for coating at 1 µg/cm <sup>2</sup> in vitronectin dilution (CellAdhere) buffer
CellAdhere™ Dilution Buffer	StemCell Technologies	07183	vitronectin dilution buffer
UltraPure™ 0.5M EDTA, pH 8.0	Thermo-Fisher	15575020	dilute with PBS to 0.5 mM before use
EVOS® FL Imaging System	Thermo-Fisher Scientific	AMF4300	LCD imaging microscope system
CKX53 Inverted Microscope	Olympus		phase contrast cell culture microscope
Pierce™ 16% Formaldehyde (w/v), Methanol-free	Thermo-Fisher	28908	dilute to 4% with PBS before use, diluted can be stored at 2-8 °C for 1 week
Perm Buffer III	BD Biosciences	558050	permeabilization buffer, chill to -20 °C before use
Mouse IgG1, κ Isotype Control, Alexa Fluor® 488	BD Biosciences	557782	isotype control for Oct3/4A, Nanog
Mouse IgG1, κ Isotype Control, Alexa Fluor® 647	BD Biosciences	557783	isotype control for Sox2
Mouse anti-human Oct3/4 (Human Isoform A), Alexa Fluor® 488	BD Biosciences	561628	
Mouse anti-human Nanog, Alexa Fluor® 488	BD Biosciences	560791	
Mouse anti-human Sox-2, Alexa Fluor® 647	BD Biosciences	562139	
Mouse IgGM, κ Isotype Control, Alexa Fluor® 488	BD Biosciences	401617	isotype control for TRA-1-60
Mouse IgGM, κ Isotype Control, Alexa Fluor® 647	BD Biosciences	401618	isotype control for TRA-1-81
Mouse anti-human TRA-1-60, Alexa Fluor® 488	BD Biosciences	330613	
Mouse anti-human TRA-1-81, Alexa Fluor® 647	BD Biosciences	330705	
Mouse IgG1, κ Isotype Control, Alexa Fluor® 488	BD Biosciences	400129	isotype control for SSEA-1
Mouse IgG3, κ Isotype Control, Alexa Fluor® 647	BD Biosciences	401321	isotype control for SSEA-4
Mouse anti-human SSEA-1, Alexa Fluor® 488	BD Biosciences	323010	

Mouse anti-human SSEA-4, Alexa Fluor® 647	BD Biosciences	330407	
Affinipure F(ab') <sub>2</sub> Fragment Goat Anti-Mouse IgG+IgM, Alexa Fluor® 488	Jackson ImmunoResearch	115-606-068	use at a dilution of 1:600 or further optimize
Affinipure F(ab') <sub>2</sub> Fragment Goat Anti-Mouse IgG+IgM, Alexa Fluor® 647	Jackson ImmunoResearch	115-546-068	use at a dilution of 1:600 or further optimize
DAPI	Thermo-Fisher Scientific	D21490	stock solution 10 mM, further dilute to 1:12.000 for a working solution
Corning® Matrigel® Growth Factor Reduced, Phenol Red-Free	Corning	356231	basement membrane matrix (BMM)
scid-beige mice, female	Taconic	CBSCBG-F	
RNeasy Plus Mini Kit (50)	Qiagen	74134	RNA isolation kit
T-25 flasks, tissue culture-treated	Thermo-Fisher	156367	
T-75 flasks, tissue culture-treated	Thermo-Fisher	156499	
Nunc™ tissue-culture dish	Thermo-Fisher	12-567-650	10 cm tissue culture dish
6-well plates, tissue-culture treated	Thermo-Fisher	140675	
Neubauer counting chamber (hemacytometer)	VWR	15170-173	
Mr. Frosty™ Freezing Container	Thermo-Fisher	5100-0001	freezing container
FACS tubes, Round Bottom Polystyrene Test Tube, 5ml	Corning	352058	5 ml polystyrene tubes
Eppendorf tubes, 1.5 ml	Thermo-Fisher	05-402-96	1.5 ml microcentrifuge tubes
PCR tubes, 200 µl	Thermo-Fisher	14-222-262	
pipette tips, 100 to 1250 µl	Thermo-Fisher	02-707-407	narrow-bore 1 mL tips
pipette tips, 5 to 300 µl	Thermo-Fisher	02-707-410	
pipette tips, 0.1 to 10 µl	Thermo-Fisher	02-707-437	
wide-bore pipette tips, 1000 µl	VWR	89049-166	wide-bore 1 mL tips
glass Pasteur pipettes	Thermo-Fisher	13-678-20A	
ethanol, 200 proof	Thermo-Fisher	04-355-451	
vortex mixer	VWR	10153-842	
chambered coverglass, 8-well, 1.5mm borosilicate glass	Thermo-Fisher	155409	glass-bottom confocal-grade cultureware
22G needles	VWR	82002-366	
insulin syringes	Thermo-Fisher	22-253-260	
Formalin solution, neutral buffered, 10%	Sigma-Aldrich	HT501128-4L	fixation of explanted teratomas
Illumina HT-12 v4 Expression BeachChip	Illumina	BD-103-0204	expression microarray, supported by PluriTest, discontinued by manufacturer
PrimeView Human Genome U219 Array Plate	Thermo-Fisher	901605	expression microarray (formerly Affymetrix brand), soon to be supported by PluriTest
GeneChip™ Human Genome U133 Plus 2.0 Array	Thermo-Fisher	902482	expression microarray (formerly Affymetrix brand), supported by CellNet, soon to be supported by PluriTest
PluriTest®	Coriell Institute		www.pluritest.org, free service for bioinformatic assessment of pluripotency, accepts microarray data - *.idat files from HT-12 v4 platform, soon to support

			U133, U219 microarray and RNA sequencing data
CellNet	Johns Hopkins University		cellnet.hms.harvard.edu, free service for bioinformatic identification of cell type, including pluripotent stem cells, based on U133 microarray data - *.cel files, soon to support RNA sequencing data