

Materials List for:

***In Vitro* Differentiation of Human Mesenchymal Stem Cells into Functional Cardiomyocyte-like Cells**

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Materials

Name	Company	Catalog Number	Comments
0.25% Trypsin/EDTA	Gibco	25200056	For cell dissociation
Alpha-MEM	Gibco	12571071	For HUCPVC and BMSC culture media.
PE-conjugated anti-human/mouse CD49f antibody	Biolegend	313612	Integrin marker for FC
APC-conjugated human Cx43/GJA1 antibody	R&D Systems	FAB7737A	Connexin 43 marker for FC
FITC-conjugated HLA-A2 antibody	Genway Biotech Inc.	GWB-66FBD2	Immunogenicity marker for FC
FITC-conjugated anti-HLA-G [MEM-G/9] antibody	Abcam	ab7904	Immunogenicity marker for FC
FITC-conjugated mouse anti-human SIRPA/CD172a antibody	AbD Serotec/Bio-Rad	MCA2518F	Cardiac marker for FC
APC-conjugated human TRA-1-85/CD147 antibody	R&D Systems	FAB3195A	Human cell marker for FC and FACS
FITC-conjugated human TRA-1-85/CD147 antibody	R&D Systems	FAB3195F	Human cell marker for FC and FACS
Anti-connexin 43/GJA1 antibody	Abcam	ab11370	Cx43. For ICC
Goat anti-rabbit IgG (H+L) cross-absorbed secondary antibody, Alexa Fluor 555	Life Technologies	A-21428	For ICC
Anti-sarcomeric alpha actinin [EA-53] antibody	Abcam	ab9465	aSARC. For ICC
Goat anti-mouse IgM heavy chain cross-absorbed secondary antibody, Alexa Fluor 555	Life Technologies	A-21426	For ICC
Mef2C (D80C1) XP rabbit antibody	New England BioLabs Ltd.	5030S	For ICC
Donkey anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor 488	Life Technologies	A-21206	For ICC
Anti-nuclei (HuNu) (clone 235-1) antibody	EMD Millipore	MAB1281	For ICC
MZ9.5 Stereomicroscope	Leica		For imaging aggregates.
1.5 ml centrifuge microtubes	Axygen	MCT-150-C	For staining MSCs with fluorescent dye.

ImageJ			Open source image processing software.
Aria II	BD		UHN SickKids FC Facility. For cell sorting.
Bone marrow mesenchymal stromal cells	Lonza	PT-2501	BMSCs
Bovine serum albumin	Sigma-Aldrich	A7030-100G	BSA. To prepare solutions for ICC
BrdU	EMD Millipore	MAB3424	Caution: BrdU is a strong teratogen and suspected mutagen. Please ensure proper training and refer to the SDS before use.
Canto II	BD		UHN SickKids FC Facility. For flow cytometry.
cDNA EcoDry Premix	Clontech/Takara	639570	For preparation of cDNA for qPCR
CellTracker Green CMFDA Dye	Life Technologies	C7025	Fluorescent imaging of cell cytoplasm
Countess automated cell counter	Invitrogen Inc.	C10227	For cell counting
DMEM-F12	Sigma-Aldrich	D6421	For rat primary cardiomyocyte culture medium.
Dulbecco's Phosphate Buffered Saline	Gibco	10010023	D-PBS, without Ca^{2+} , Mg^{2+}
EVOS	Life Technologies		In-house fluorescent microscope
FACSCalibur	BD		In-house. For flow cytometry.
Fetal bovine serum (Hyclone)	GE Healthcare	SH3039603	FBS. Component of cell culture medium.
IDT Prime Time qPCR probes	Integrated Data Technologies	FAM fluorophore	http://www.idtdna.com/pages/products/gene-expression/primetime-qpcr-assays-and-primers
Lab Vision PermaFluor Aqueous Mounting Medium	ThermoScientific	TA-030-FM	For storage of cells to undergo ICC
LSR II	BD		UHN SickKids FC Facility. For flow cytometry.
MoFlo Astrios	Beckman Coulter		UHN SickKids FC Facility. For cell sorting.
Normal goat serum	Cell Signaling Technology	5425S	NGS. Used in blocking solution for ICC
Nunc Lab-Tek II Chamber Coverglass, 8-wells	Thermo Scientific Nunc	155409	To prepare samples for ICC
OmniPur Triton X-100 Surfactant	EMD Millipore	9410-OP	As a component of permeabilizing solution when preparing cells for ICC
Paraformaldehyde, 16% Solution, EM Grade	Electron Microscopy Sciences	15710	For fixing cells for ICC.
Penicillin/streptomycin	Gibco	15140122	Component of cell culture medium.
Primers	Sigma	Custom Standard DNA Oligos, Desalted, 0.2 μmol	CTnT_F : GGC AGC GGA AGA GGA TGC TGA A; CTnT_R : GAG GCA CCA AGT TGG GCA TGA ACG A; MYH6 F : GCA AAG TAC TGG ATG ACA CGC T; MYH6 R : GTC ATT GCT GAA ACC GAG AAT G
Quorum Spinning Disk Confocal	Zeiss		SickKids Imaging Facility
ReproCardio hiPS cell derived cardiomyocytes	ReproCell	RCD001N	Positive control for qPCR

RNeasy mini kit	Qiagen	74106	To isolate RNA for qPCR
Rotor-Gene SYBR Green PCR Kit	Qiagen	204074	For qPCR with master mix
RPMI 1640	Gibco	A1049101	For MSC, monocyte coculture medium.
TaqMan qPCR primer assays	Thermo Fisher Scientific	4444556	For qPCR
Trypan Blue	Life Technologies	T10282	Staining of cells for viability and counting
Trypsin	Gibco	272500108	For cell dissociation
Volocity	Perkin-Elmer	Volocity 6.3	Imaging software
0.2 µm pore filter	Thermo Fisher Scientific	566-0020	For sterilizing tissue culture media
HERAcell 150i CO ₂ Incubator	Thermo Fisher Scientific	51026410	For incubating cells
Dulbecco's phosphate buffered saline	Sigma-Aldrich	D8537	PBS. 1X, Without calcium chloride and magnesium chloride
Forceps	Almedic	7727-A10-704	For handing rat heart. Can use any similar forceps.
Scissors	Fine Science Tools	14059-11	For mincing rat heart. Curved scissors recommended.
50 mL tube	BD Falcon	352070	For collection during cardiomyocyte collection and general tissue culture procedures
15 mL tube	BD Falcon	352096	For general tissue culture procedures
6-well plates	Thermo Scientific Nunc	CA73520-906	For tissue culture
10 cm tissue culture dishes	Corning	25382-428	For aggregate formation
Axiovert 40C Microscope	Zeiss		For bright-field imaging through out tissue culture and the rest of the protocol
70 µm cell strainer	Fisherbrand	22363548	To ensure a single cell suspension before flow cytometry or sorting
Triton X-100	EMD Millipore	9410-1L	Used in permeabilization solution for ICC
Hoechst 33342	Thermo Fisher Scientific	H1399	Stain used during visualization of Cx43 localization