

Materials List for:

Induction of Endothelial Differentiation in Cardiac Progenitor Cells Under Low Serum Conditions

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Materials

Name	Company	Catalog Number	Comments
Culture medium			
Iscove's Modified Dulbecco's Medium (IMDM)	ThermoFisher	#12440	
Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient Mixture F12 Ham	Merck	#D8437	
Penicillin-Streptomycin (P/S)	ThermoFisher	#15140122	
Fetal Bovine Serum (FBS)	Hyclone	#SH30071	3.5% (0.1% for lineage induction)
L-Glutamine	ThermoFisher	#25030	Final concentration 2 mM
Glutathione	Merck	#G6013	
Recombinant Human Epidermal Growth Factor (EGF)	Peprtech	#AF-100-15	
Recombinant Basic Fibroblast Growth Factor (FGF)	Peprtech	#AF-100-18B	
B27 Supplement	ThermoFisher	#17504044	
Cardiotrophin 1	Peprtech	#250-25	
Thrombin	Diagontech AG, Switzerland	#100-125	
Hanks' Balanced Salt Solution (HBSS) CaCl ₂ (-), MgCl ₂ (-)	ThermoFisher	#14170	
0.05 % Trypsin-EDTA	ThermoFisher	#25300	
T75 Flask	Sarstedt	#83.3911	
Endothelial differentiation			
Endothelial Cell Growth Medium (EGM)-2 BulletKit	Lonza	#CC-3162	
Ham's F-12K (Kaighn's) Medium	ThermoFisher	#21127	
Laminin	Merck	#L2020	
Fibronectin	Merck	#F4759	Dilute in ddH ₂ O
6 Well Plate	Falcon	#353046	
Formaldehyde Solution	Merck	#F1635	Dilute 1:10 in PBS (3.7% final concentration)
Triton X-100	Merck	#93420	0.1% in ddH ₂ O
Normal Goat Serum (10%)	ThermoFisher	#50062Z	
Anti-von Willebrand Factor antibody	Abcam	#ab6994	1:100 in 10% goat serum

Goat anti-Rabbit IgG, Alexa Fluor 546	ThermoFisher	#A11010	1:500 in 10% goat serum
4',6-diamidino-2-phenylindole, dihydrochloride (DAPI)	ThermoFisher	#62247	1:500 in ddH2O
SlowFade Antifade Kit	ThermoFisher	#S2828	
BX63 widefield microscope	Olympus		
Tube formation			
96 Well Plate	Falcon	#353072	
5 ml Round Bottom Tube with Strainer Cap	Falcon	#352235	
Matrigel Growth Factor Reduced	Corning	#354230	
IX50 widefield microscope	Olympus		
Sca-1+/CD31- cardiac side population isolation³⁴			
Reagents			
Pentobarbital Natrium 50 mg/ml ad usum vet.	in house hospital pharmacy	#9077862	Working solution: 200 mg/kg
Phosphate Buffered Saline (PBS) CaCl ₂ (-), MgCl ₂ (-)	ThermoFisher	#20012	
Hanks' Balanced Salt Solution (HBSS) CaCl ₂ (-), MgCl ₂ (-), phenol red (-)	ThermoFisher	#14175	Prepare HBSS 500 mL + 2% FBS for quenching Collagenase B activity
Dulbecco's Modified Eagle's Medium (DMEM) 1g/L of D-Glucose, L-Glutamine, Pyruvate	ThermoFisher	#331885	Prepare DMEM + 10% FBS + 25 mM HEPES+ P/S for Hoechst staining
Penicillin-Streptomycin (P/S)	ThermoFisher	#15140122	
HEPES 1 M	ThermoFisher	#15630080	Final concentration 25 mM
Fetal Bovine Serum (FBS)	Hyclone	#SH30071	
RBC LysisBuffer (10X)	BioLegend	#420301/100mL	Dilute to 1X in ddH2O and filter through a 0.2 µm filter
Collagenase B	Merck	#11088807001	Final concentration 1 mg/mL in HBSS, filtered through a 0.2 µm filter
bisBenzimide H33342 Trihydrochloride (Hoechst)	Merck	#B2261	Prepare 1 mg/mL in ddH2O
Verapamil-hydrochloride	Merck	#V4629	Final concentration 83.3 µM
APC Rat Anti-Mouse CD31	BD Biosciences	#551262	0.25 µg/10 ⁷ cells
FITC Rat Anti-Mouse Ly-6A/E (Sca-1)	BD Biosciences	#557405	0.6 µg/10 ⁷ cells
7-Aminoactinomycin D (7-ADD)	ThermoFisher	#A1310	0.15 µg/10 ⁶ cells
APC rat IgG2a k Isotype Control	BD Biosciences	#553932	0.25 µg/10 ⁷ cells
FITC Rat IgG2a k Isotype Control	BD Biosciences	#554688	0.6 µg/10 ⁷ cells
Material			
Needles 27G	Terumo	#NN-2719R	
Needles 18G	Terumo	#NN-1838S	
Single Use Syringes 1 mL sterile	CODAN	#62.1640	
Transferpipette 3.5 mL	Sarstedt	#86.1171.001	
Cell Strainer 40 µm blue	BD Biosciences	#352340	
Cell Strainer 100 µm yellow	BD Biosciences	#352360	
Lumox Dish 50	Sarstedt	#94.6077.305	

Culture Dishes P100	Corning	#353003	
Culture Dishes P60	Corning	#353004	
Mouse			
Line	Age	Breeding	
C57BL/6NRj / male	12 weeks	in house	
Product Name	Company	Catalogue No.	
Reagents			
Iscove's Modified Dulbecco's Medium (IMDM)	ThermoFisher	#12440	
Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient Mixture F12 Ham	Merck	#D8437	
Penicillin-Streptomycin (P/S)	ThermoFisher	#15140122	
Fetal Bovine Serum (FBS)	Hyclone	#SH30071	
L-Glutamine	ThermoFisher	#25030	
Glutathione	Merck	#G6013	
B27 Supplement	ThermoFisher	#17504044	
Recombinant Human Epidermal Growth Factor (EGF)	Peprtech	#AF-100-15	
Recombinant Basic Fibroblast Growth Factor (FGF)	Peprtech	#AF-100-18B	
Cardiotrophin 1	Peprtech	#250-25	
Thrombin	Diagontech AG, Switzerland	#100-125	
Endothelial Cell Growth Medium (EGM)-2 BulletKit	Lonza	#CC-3162	
Overview of medium compositions. Some of this information is identical with the one provided above, but sorted according to the composition of Media 1-3.			
Product Name	Medium 1⁸	Medium 2	Medium 3
Reagents	Culture	Lineage induction	Endothelial diff.
Iscove's Modified Dulbecco's Medium (IMDM)	35%	35%	
Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient Mixture F12 Ham	65%	65%	
Penicillin-Streptomycin (P/S)	1%	1%	
Fetal Bovine Serum (FBS)	3.5%	≤0.1%	
L-Glutamine	2 mM	2 mM	
Glutathione	0.2 nM	0.2 nM	
B27 Supplement	1.3%		
Recombinant Human Epidermal Growth Factor (EGF)	6.5 ng/mL		
Recombinant Basic Fibroblast Growth Factor (FGF)	13 ng/mL		
Cardiotrophin 1	0.65 ng/mL		
Thrombin	0.0005 U/mL		All necessary components included in the kit

Endothelial Cell Growth Medium (EGM)-2 BulletKit			All necessary components included in the kit
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