

Materials List for

An Antegrade Perfusion Method for Cardiomyocyte Isolation from Mice

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Materials

Name	Company	Catalog Number	Comments
Amphotericin B	Wako Pure Chemical Industries, Japan		
Alexa Fluor 488 anti-mouse IgG antibody	Molecular Probes, USA	A11001	Fluorescent-labeled secondary antibody. (1:400 dilution for immunostaining)
Anti- α -actinin (ACTN)	Sigma-Aldrich, USA	A7811	Mouse monoclonal antibody (clone EA-53). (1:400 dilution for immunostaining)
Anti-atrial natriuretic peptide (ANP)	Merck-Millipore, USA	AB5490-I	Rabbit polyclonal antibody (1:2000 dilution for Western blots)
Anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Cell Signaling Technology, USA	2118	Mouse monoclonal antibody (1:10000 dilution for Western blots)
Anti-smooth muscle actin (SMA)	Dako, Denmark	M0851	Mouse monoclonal antibody (clone 1A4) (1:400 for immunostaining)
Anti-rabbit IgG antibody	Amersham, GE Healthcare, USA	NA934	Secondary antibody (1:10000 dilution for Western blots)
ATP disodium salt	Sigma-Aldrich, USA	A26209	
Bovine serum albumin (BSA)	Sigma-Aldrich, USA	A9418	
Cell-Tak	Corning	354240	Biological material for adhesion of the cell or tissues
Chemi-Lumi One Super	Nacalai Tesque, Japan	02230-14	Chemiluminescent reagent used for western blotting.
Collagenase Type 2	Worthington Biochemicals, USA	LS004176	Choose the activity guaranteed to be greater than 300 unit/mg.
Complete Mini	Roche, Germany	11836153001	A mixture of several protease inhibitors.
4'6'diamidino-2-phenylindole (DAPI)	Nacalai Tesque, Japan	11034-56	Used for cell-impermeant nuclear staining
Dulbecco's Modified Eagle's Medium (DMEM)	Nacalai Tesque, Japan	08458-45	including 4.5 g/L glucose
Extension tube	Top, Japan	X1-50	Connect with syringe and injection needle for antegrade perfusion.
EPC-8 patch-clamp amplifier	HEKA, Germany		

Fetal bovine serum (FBS)	Sigma-Aldrich, USA	F7524-500ML	
Glass capillaries	Narishige Scientific Instrument Lab., Japan		outside diameter 1.5 mm, inside diameter 0.9 mm
GTP lithium salt	Sigma-Aldrich, USA	G5884	
Horizontal microelectrode puller	Germany)	P-97	
Heater mat	Natsume Seisakusho, Japan	KN-475-3-40	Equipment to warm the perfusion plate.
Infusion pump	TERUMO, Japan	TE-311	Infusion syringe pump for antegrade perfusion.
Injeciton needle (27 gauge)	TERUMO, Japan	NN-2719S	Needle for insertion into the left ventricle.
Insulin (from bovine pancrease)	Sigma-Aldrich, USA	I5500	Dissolve in 0.1 M HCl.
Mini cordless grinder	Funakoshi, Japan	cG-4A	Small grinder for homogenizing tissue in 1.5 mL sample tube.
4%-Paraformaldehyde Phosphate Buffer solution (4% PFA)	Nacalai Tesque, Japan	09154-85	
Penicillin G potassium	Nacalai Tesque, Japan	26239-84	
Phenol Red	Nacalai Tesque, Japan	26807-21	
10X Phosphate Buffered Saline (pH7.4) (10X PBS)	Nacalai Tesque, Japan	27575-31	
Plastic multi-well culture plate	Falcon, USA	353226	Use the lid of the multi-well culture plate as the perfusion plate.
Plastic syringe (20 mL)	TERUMO, Japan	SS-20ES	Use for infusion of CIB-EGTA.
Plastic syringe (30 mL)	TERUMO, Japan	SS-30ES	Use for infusion of Enzyme-mix
Plastic transfer pipette	Sarstedt, Germany	86.1171	Cut the tip just before sucking mouse heart into the pipette.
Polyvinylidene difluoride (PVDF) membrane	Merck-Millipore, USA	IPVH00010	Immobilin-P membrane (Transfer membrane for protein blotting)
Protease	Sigma-Aldrich, USA	P5147	A mixture of three or more proteases including extracellular serine protease.
4X Sample buffer solution	Fuji Film, Japan	198-13282	Contains 0.25 M Tris-HCl (pH 6.8), 8 w/v% SDS, 40 w/v% Glycerol and 0.02 w/v% BPB
SDS polyacrylamide gel (15%)	Fuji Film, Japan	193-14991	
Streptomycin sulfate	Nacalai Tesque, Japan	32237-14	
10X Tris-Glycine buffer solution (10X TG)	Nacalai Tesque, Japan	09422-81	Contains 0.25 M-Tris and 1.92 M-Glycine, (pH 8.3)
Trypsin	Sigma-Aldrich, USA	T8003	Trypsin from bovine Type 1.
Vascular clamp	Karl Hammacher GmbH, Germany	HSE 004-35	Small straight vascular clamp used for clamping aorta.
All other reagents	Nacalai Tesque, Japan		