Materials List for:

Live Cell Imaging of Primary Rat Neonatal Cardiomyocytes Following Adenoviral and Lentiviral Transduction Using Confocal Spinning Disk Microscopy

Takashi Sakurai[1,2], Anthony Lanahan[2], Melissa J. Woolls[2], Na Li[2], Daniela Tirziu[2], Masahiro Murakami[2]

[1]Max-Planck-Institute for Molecular Biomedicine and Institute of Cell Biology

[2]Department of Internal Medicine, Yale Cardiovascular Research Center and Section of Cardiovascular Medicine

Correspondence to: Takashi Sakurai at takashi.sakurai@mpi-muenster.mpg.de

URL: https://www.jove.com/video/51666
DOI: doi:10.3791/51666

Materials

Name	Company	Catalog Number	Comments
1 Scissors for decapitation	WPI	501749	Autoclave before use
1 Fine scissors for heart isolation and chopping	WPI	14393	Autoclave before use
2 Fine forceps (Dumont No. 5)	Sigma	F6521	Autoclave before use
3 Sterilized 10 cm plastic dishes	Sigma	CLS430165	For heart isolation
3.5 cm Glass bottom culture dishes	MatTek	P35G-1.5-20-C	For final plating of cardiomyocytes for future live cell imaging. Micro-Dishes from ibidi are an acceptable alternative.
3.5 cm Glass bottom culture dishes	MatTek	P35G-0.17-14-C	For TIRF or high resolution image
Ethanol solution, 70% (v/v) in water	Sigma	E7148	
2% Gelatin	Sigma	G1393	
1 Sterilized 10 cm plastic dish	Sigma	CLS430165	For trypsinization
Aluminium foil	Any brand		
Parafilm	Sigma	P7543	
Two 10 cm plastic cell culture dish	Sigma	CLS430165	For selection
Autopipette	Drummond Scientific	4-000-300	For trituration
Cell counter			
Cellometer, automated cell counter	nexcelom		To check and count cells
Microscope and hematocytometer	Any brand		To check and count cells
Trypan blue solution, 0.4%	invitrogen	15250-061	
CO_2 incubator	Sanyo	MCO-19AIC	
Incubating orbital shaker	Sigma	Z673129	To shake heart tissue with collagenase at 37 °C at 170-200 rpm
10 mg/ml BrdU solution	BD Pharmingen	550891	
DMEM, high glucose	invitrogen	41965039	Mix medium as indicated in the protocol and warm before use
MEM	invitrogen	31095029	Mix medium as indicated in the protocol and warm before use
Fetal bovine serum	invitrogen	26140079	
Penicillin-streptomycin (10,000 U/ml)	invitrogen	15140-122	
Section 2 Lentiviral transduction			
3 Packaging plasmids			

pMDLg/pRRE	Addgene	12251	
pRSV-Rev	Addgene	12253	
pMD2.G	Addgene	12259	
The lentiviral transfer vector, pLVX-IRES-Puro	Clontech	632183	
Opti-MEM (serum-free medium)	invitrogen	31985070	
Transfection reagent			
polyethyleneimine“Max”, (MW 40,000) - High Potency Linear PEI (Equivalent to Mw 25,000 in Free Base Form)	Polysciences	24765-2	It can be substituted with X-tremeGENE 9 from Roche
X-tremeGENE 9	Roche	6365779001	substitute for PEI as transfection reagent
Chloroquine	Sigma	C6628	Dissolve in water and make 100 mM stock solution. Inhibition of endosomal acidification can be achieved with 10-100 µM Chloroquine.
HEPES	Sigma	H3375	
10 ml Luer-Lok syringe, sterilized	BD	309604	
0.45 µm filters	Sigma	F8677	Use only cellulose acetate or polyethersulfone (PES) (low protein binding) filters. Do not use nitrocellulose filters. Nitrocellulose binds surface proteins on the lentiviral envelope and destroys the virus.
Hexadimethrine bromide	Sigma	H9268	Dissolve in water and make 8 mg/ml stock solution, then filter it to sterilize.
Polyethylene glycol 6,000	Sigma	81260	
Sodium chloride	Sigma	S9888	
Sodium hydroxide	Sigma	S5881	
Section 3 Adenoviral transduction			
HEK 293T cells	ATCC	CRL-11268	
Some 10 cm cell culture dishes	Sigma	CLS430165	
96-Well microplate with lid, flat-bottom, tissue culture, sterile	BD Falcon	353072	For titration
Multichannel pipette, 10-100 µl, 8-channel	eppendorf	3122 000.035	
Section 4 Live cell imaging			
Spinning disk confocal microspopy	PerkinElmer	L7267000	
A temperature-controlled chamber	Any brand		To keep temperature at 37 °C
A CO_2 environmental system	Any brand		Optional to maintain CO_2 concentration optimal
CO_2 Independent medium, no glutamine	invitrogen	18045-054	For long time time-lapse imaging
DMEM, high glucose, HEPES, no phenol red	invitrogen	21063-029	For long time time-lapse imaging