

Materials List for:

Isolation and Characterization of Primary Rat Valve Interstitial Cells: A New Model to Study Aortic Valve Calcification

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URL: <https://www.jove.com/video/56126>

DOI: [doi:10.3791/56126](https://doi.org/10.3791/56126)

Materials

Name	Company	Catalog Number	Comments
Dissection scissors	World Precision Instruments	14393	Autoclave before use.
Spring straight (8 mm blade)	World Precision Instruments	15905	Autoclave before use.
SuperFine Vannas scissors (5 mm blade, 8 cm)	World Precision Instruments	14003	Autoclave before use.
Dumont #5 tweezers (11 cm)	World Precision Instruments	500342	Autoclave before use.
DMEM-F12	Thermo Fisher Scientific	11320074	
Foetal bovine serum	Thermo Fisher Scientific	10500064	Filter before adding to culture medium.
Gentamicin	Thermo Fisher Scientific	15710049	
Absolute ethanol	Thermo Fisher Scientific	E/0650DF/17	Dilute to 70% v/v in distilled water.
Hydrochloric acid (HCl)	Thermo Fisher Scientific	H1150PB17	Dilute to 0.6M with distilled water.
Sodium hydroxide (NaOH)	Thermo Fisher Scientific	UN1823	Dilute to 0.1M with distilled water.
DAPI	Thermo Fisher Scientific	P36931	
Alexa Fluor 594 goat anti-mouse antibody	Thermo Fisher Scientific	A11005	1/250 dilution in antibody dilution buffer.
Alexa Fluor 488 donkey anti-rabbit antibody	Thermo Fisher Scientific	A21206	1/250 dilution in antibody dilution buffer.
Superscript II kit	Thermo Fisher Scientific	18064014	
dNTP	Thermo Fisher Scientific	18427013	
Random primers	Thermo Fisher Scientific	P/N58875	
Taq Polymerase kit	Thermo Fisher Scientific	18038026	
Tris/Borate/EDTA (TBE)	Thermo Fisher Scientific	AM9863	Dilute to 1x with distilled water, before use.
Agarose	Thermo Fisher Scientific	16500	2% in 1x Tris/Borate/EDTA(TBE).
Lysis buffer (RIPA)	Thermo Fisher Scientific	89900	
T75 flask	Thermo Fisher Scientific	156472	
T175 flask	Thermo Fisher Scientific	159920	
qPCR plates	Thermo Fisher Scientific	AB0990	
Rat Msx2 primer	Qiagen	QT01084090	
Rat Alpl primer	Qiagen	QT00190680	
Rat Enpp1 primer	Qiagen	QT00181426	
RNeasy minikit	Qiagen	74104	
6-well plates	Sigma	CLS3516	
T25 flask	Sigma	CLS430639	

Monobasic phosphate	Sigma	S5011	
Dibasic phosphate	Sigma	S5136	
Calcium chloride	Sigma	C1016	
Sodium dodecyl sulphate (SDS)	Sigma	5030	Dilute to 0.1% with 0.1M NaOH.
Paraformaldehyde	Sigma	P6148	Dilute to 4% with PBS.
Triton X100	Sigma	X100	
Bovine serum albumin (BSA)	Sigma	A3059	
Alizarin red S	Sigma	A5533	Make up to 2% with distilled water, and adjust to pH 4.2.
TGFβ-1 primer pair: f: GCTACCATGCCAACTTCTGT r: TGTGTTGGTTGTAGAGGGCA	Sigma		Concentration used: 10 μM
TGFβ-2 primer pair: f: GAAGGCAGAGTTCAGGGTCT r: CGCTGGGTTGGAGATGTTAG	Sigma		Concentration used: 10 μM
Monoclonal Anti-β-Actin -Peroxidase antibody produced in mouse	Sigma	A3854	1/50000 dilution in 5% BSA in tris-buffered saline + 0.1% Tween (TBS/T).
Mouse anti-vimentin antibody	Sigma	v6389	1/900 dilution in antibody dilution buffer (1x phosphate buffered saline (PBS), 1% bovine serum albumin (BSA), 0.3% Triton X-100).
Mouse IgG	Sigma	I5381	Diluted to the same stock concentration as mouse anti-vimentin antibody prior to dilution in antibody dilution buffer.
Rabbit IgG	GeneTex	GTX35035	Diluted to the same stock concentration as rabbit anti-α-smooth muscle actin antibody prior to dilution in antibody dilution buffer.
Rabbit anti-α-smooth muscle actin antibody	Abcam	ab5694	1/200 dilution in antibody dilution buffer.
Rabbit anti-CD31	Abcam	ab28364	1/50 dilution in 5% BSA in TBS/T.
HRP-conjugated goat anti-rabbit antibody	Dako	P0448	1/3000 dilution in in 5% BSA in TBS/T.
Collagenase II	Worthington	41512862	Adjust to 425 U/mL with distilled water, and then filter.
SYBR green mastermix	Primerdesign	PrecisionPLUS-MX-SY	
Calcium assay kit	Randox	CA590	
DC protein assay kit	Bio-Rad	5000111	
ECL reagent	GE Healthcare	RPN2109	
Hyperfilm ECL	GE Healthcare	28906837	
Nitrocellulose membrane	GE Healthcare	10600007	
PCR ladder	Bioline	BIO-33057	
Loading dye for gel electrophoresis	New England Biolabs	B7025S	
Syringe filters (0.22 μm)	Merck Millipore	SLGP033RS	
Coverslips	Scientific Laboratory Supplies LTD	MIC3100	
Hematocytometer	Marienfeld Superior	640030	
Name	Company	Catalog Number	Comments
Camera set up for Alizarin red images:			
Camera	Nikon D800		

Lens	Nikon AF_s Micro, Nikko 105 mm, 1:2.8 GED		
Dissection forceps 10 cm, curved ends	World Precision Instruments	15915	Autoclave before use.