

Materials List for:

Analysis of Tubular Membrane Networks in Cardiac Myocytes from Atria and Ventricles

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Materials

Name	Company	Catalog Number	Comments
Chemicals and Enzymes			
2,3-Butanedione monoxime	Sigma-Aldrich, Munich, Germany	B0753	
Bovine calf serum	Thermo Scientific, Schwerte, Germany	SH30073	Triple 0.1 µm sterile filtered.
CaCl ₂	Sigma-Aldrich, Munich, Germany	21115	Diluted 1:10 in MQ water to obtain 100 mM CaCl ₂ stock concentration.
Collagenase type II	Worthington via Cell Systems, Troisdorf, Germany	on request	Enzymatic activity depends on individual collagenase batches. Collagenase II and other enzyme activities (Caseinase, Clostripain, Tryptic) can be assessed in the "collagenase lot selection tool". Determine cell yield and quality individually for each new lot of collagenase.
Glucose	Carl Roth, Karlsruhe, Germany	HN06.1	
Heparin	Rotexmedica, Trittau, Germany	PZN-03862340	Diluted in 0.9% NaCl and injected subcutaneously in abdominal skin.
HEPES	Carl Roth, Karlsruhe, Germany	9105.4	
Forene 100% (V/V)	Abbott, Libertyville, IL, USA	B506	Active agent: isoflurane, 250 ml. Use approximately 2 Vol% in air/oxygen dispenser instrument.
KCl	Carl Roth, Karlsruhe, Germany	6781.3	
KH ₂ PO ₄	Carl Roth, Karlsruhe, Germany	3904.2	
Laminin (2 mg/ml)	BD Biosciences, Heidelberg, Germany	354232	Lamination is described under step 2.1.
MgCl ₂ ·6H ₂ O	Carl Roth, Karlsruhe, Germany	2189.2	
MgSO ₄ ·7H ₂ O	Carl Roth, Karlsruhe, Germany	8283.2	
Na ₂ HPO ₄ ·2H ₂ O	Carl Roth, Karlsruhe, Germany	4984.2	
NaHCO ₃	Carl Roth, Karlsruhe, Germany	HN01.1	
Taurin	Carl Roth, Karlsruhe, Germany	4721.2	
Dyes			
Di-8-ANEPPS	Molecular Probes, Life Technologies, Darmstadt, Germany	D-3167	Stock solution 2 mM in DMSO
Trypan blue	Sigma-Aldrich, Munich, Germany	T8154	Trypan blue is gently mixed 1:1 via tip-cut 1 ml plastic pipette with cell

			suspension prior to cell counting in Neubauer cytometer.
Langendorff Perfusion Setup			
Circulation thermostat	Lauda, Lauda-Königshofen, Germany		Please refer to Louch <i>et al.</i> (JMCC 2011). Heat up thermostat and buffers in perfusion tubing to 37 °C 15 min prior to use.
Flexible silicone tubing Tygon for peristaltic pump	VWR, Darmstadt, Germany	224-2252	Tubing needs to be changed regularly.
Flexible silicone tubing Tygon for thermostat	VWR, Darmstadt, Germany	228-4340	
Heating coil surrounding perfusion tubing	Rettberg, Göttingen, Germany	custom-made	Heating coil and tubing needs to be cleaned thoroughly via MQ water after using. Do not use detergents. Glass components should be bathed regularly in 10 mM NaOH overnight.
Peristaltic pump	Ismatec, Wertheim, Germany	ISM830	
Three way stop cock Discofix C Luer Lock 10 cm	Braun, Melsungen, Switzerland	16500C	
Three way stop cock Discofix 3SC	Braun, Melsungen, Switzerland	4095146	
Instruments			
42 mm glass coverslips	Menzel Gläser via Thermo Scientific, Schwerte, Germany	on request	0.13-0.16 mm thickness
Cannula 21 G	Becton, Dickinson and Company, Franklin Lake, NY, USA	304432	Cut to a length of ~5 mm, roughened with sandpaper.
Coverslips for Neubauer cytometer 24 x 24 mm	Menzel Gläser via Thermo Scientific, Schwerte, Germany	on request	0.38-0.42 mm thickness
Graefe forceps, 0.5 mm tips, slight curve	Fine Science Tools, Heidelberg, Germany	11151-10	
LSM 710 NLO	Carl Zeiss, Jena, Germany		63X 1.4 NA oil objective
Neubauer improved cytometer	Labor Optik, Friedrichsdorf, Germany	1100000	Counting procedure: Wipe cytometer and coverslip provided with the counting chamber with 70 % ethanol. Press coverslip gently on the counting chamber so that the two glass surfaces are in contact and Newton's rings can be observed. Subsequently, 10-20 µl cell suspension can be applied to the edge of the coverslip to be sucked into the void by capillary action. Count the intact vs. defect myocytes using the squares of the cytometer grid which reflects 100 nl. Repeat counting procedure on the second grid provided on the cytometer. Calculate the density of cells in your original cell suspension by taking account of any dilutions and counting shortcuts.
POC-R2 Imaging Chamber	Pecon, Erbach, Germany		Cell suspension volume: 800 µl; desired plating density: ~1,000 AM and ~10,000 VM
Spring scissors, 8 mm blades straight, blunt	Fine Science Tools, Heidelberg, Germany	15025-10	
Student dumont #7 forceps, inox	Fine Science Tools, Heidelberg, Germany	91197-00	

Student iris scissors, curved, 11.5 cm	Fine Science Tools, Heidelberg, Germany	91461-11	
Student iris scissors, straight, 11.5 cm	Fine Science Tools, Heidelberg, Germany	91460-11	
Student surgical scissors, straight, sharp, 12 cm	Fine Science Tools, Heidelberg, Germany	91402-12	
Tissue forceps, 1 x 2 teeth, slim, 10 cm	Fine Science Tools, Heidelberg, Germany	11023-10	