

Analysis of Cardiac Contractile Dysfunction and Ca^{2+} Transients in Rodent Myocytes

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Materials

Name	Company	Catalog Number	Comments
MEDIA			
Bovine serum albumin	Sigma (Roche)	3117057001	Final concentration = 0.2% (w/v)
Glutathione	Sigma	G-6529	Final concentration = 10 mM
HEPES	Sigma	H-7006	Final concentration = 15 mM
M199	Sigma	M-2520	1 bottle makes 1 L; pH 7.45
NaHCO_3	Sigma	S-8875	Final concentration = 4 mM
Penicillin/streptomycin	Fisher	15140122	Final concentration = 100 U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin
REAGENTS SPECIFICALLY FOR Ca^{2+} IMAGING			
Dimethylsulfoxide (DMSO)	Sigma	D2650	
Fura-2AM	Invitrogen (Molecular Probes)	F1221	50 $\mu\text{g/vial}$; Prepare stock solution of 1 mM Fura-2AM + 0.5 M probenidic in DMSO; Final Fura2-AM concentration in media is 5 μM
Probenidic	Invitrogen (Fisher)	P36400	Add 7.2 mg probenidic (0.5 M) to 1 mM Fura-2AM stock; Final concentration in media is 2.5 mM
MATERIALS FOR RAT MYOCYTE PACING			
#1 22 mm ² glass coverslips	Corning	2845-22	
3 x 36 inch cables with banana jacks	Pomona Electronics	B-36-2	Supplemental Figure 1, panel C
37°C Incubator with 95% O ₂ :5% CO ₂	Forma	3110	Supplemental Figure 1, panel E. Multiple models are appropriate
Class II A/B3 Biosafety cabinet with UV lamp	Forma	1286	Multiple models are appropriate
Forceps - Dumont #5 5/45	Fine Science Tools	11251-35	
Hot bead sterilizer	Fine Science Tools	1800-45	
Low magnification inverted microscope	Leica	DM-IL	Position this microscope adjacent to the incubator to monitor paced myocytes for contraction at the

			start of pacing and after media changes; 4X and 10X objectives recommended
Pacing chamber	Custom		Supplemental Figure 1, panel A. The Ionoptix C-pace system is a commercially available alternative or see ²²
Stimulator	Ionoptix	Myopacer	Supplemental Figure 1, panel D.
MATERIALS FOR CONTRACTILE FUNCTION and/or Ca²⁺ IMAGING ANALYSIS			ID in Supplemental Figure 2 & Alternatives/Recommended Options
Additional components for Ca ²⁺ imaging analysis	Ionoptix	Essential system components: -- Photon counting system -- Xenon power supply with dual excitation light source -- Fluorescence interface	<ul style="list-style-type: none"> - The photon counting system contains a photomultiplier (PMT) tube and dichroic mirror and is installed adjacent to the CCD camera (panel A #4). - The power supply for the xenon bulb light source (see panel A #5 and panel C, left) is integrated with a dual excitation interface (340/380 nm excitation and 510 nm emission) shown in panel A #6. - The fluorescence interface between the computer and light source is shown in panel B, #12.
CCD camera with image acquisition hardware and software (240 frames/s)	Ionoptix	Myocam with CCD controller	Myocam and CCD controller are shown in Supplemental Fig. 2, panel A #4 and panel A #5 & panel C #5 (right), respectively. The controller is integrated with a PC computer system (panel B #14).
Chamber stimulator	Ionoptix	Myopacer	Panel B, #13; Alternative: Grass model S48
Coverslip mounted perfusion chamber	Custom chamber for 22 mm ² coverslip with silicone adapter and 2-4 Phillips pan-head #0 screws (arrow, panel F)		Panel A #10 & panel F; Chamber temperature is calibrated to 37°C using a TH-10Km probe and the TC ² BIP temperature controller (see temperature controller). Commercial alternatives: Ionoptix FHD or C-stim cell chambers; Cell MicroControls culture stimulation system
Dedicated computer & software for data collection and analysis of function/Ca ²⁺ transients	Ionoptix	PC with IonWizard PC board and software	Panel B, #14; Contractile function is measured using either SarcomLen (sarcomere length) or SoftEdge (myocyte length) acquisition modules of the IonWizard software. The IonWizard software also includes PMT acquisition software for ratiometric Ca ²⁺ imaging in Fura-2AM-loaded myocytes. - A 4 post electronic rack mount cabinet and shelves are recommended for housing the computer and cell stimulator. The fluorescence interface for Ca ²⁺ imaging also is housed in this cabinet (see below).
Forceps - Dumont #5 T1	Fine Science Tools	11252-40	Panel F
Insulated tube holder for media	Custom		Panel A #9; This holder is easily assembled using styrofoam & a pre-heated gel pack to keep media warm

Inverted brightfield microscope	Nikon	TE-2000S	Install a rotating turret for epi-fluorescence (Panel A #2) for Ca ²⁺ imaging. A deep red (590 nm) condenser filter also is recommended to minimize fluorescence bleaching during Ca ²⁺ imaging.
Isolator Table	TMC Vibration Control	30 x 36 inches	Panel A, #1; Desirable: elevated shelving, Faraday shielding
Microscope eyepieces & objective	Nikon	10X CFI eyepieces 40X water CFI Plan Fluor objective	Panel A #3; 40X objective: n.a. 0.08; w.d. 2 mm. A Cell MicroControls HLS-1 objective heater is mounted around the objective (see temperature controller below). NOTE: water immersion dispensers also are now available for water-based objectives.
Peristaltic pump	Gilson	Minipuls 3	Panel A #8 and panel E
small weigh boat	Fisher	08-732-112	
Temperature controller	Cell MicroControls	TC ² BIP	Panel A #7; Panel D. This temperature controller heats the coverslip chamber to 37°C. A preheater and objective heater are recommended for this platform. A Cell MicroControls HPRE2 preheater and HLS-1 objective heater are controlled by the TC ² BIP temperature controller for our studies.
Under cabinet LED light with motion sensor	Sylvania	#72423 LED light	Recommended for data collection during Ca ²⁺ transient imaging under minimal room light.. Alternative: A clip on flashlight/book light with flexible neck - multiple suppliers are available.
Vacuum line with in-line Ehrlenmeyer flask & protective filter	Fisher	Tygon tubing - E363; polypropylene Ehrlenmeyer flask - 10-182-50B; Vacuum filter - 09-703-90	Panel A #11