Isolation of cardiac myocytes and measurement of myocyte shortening

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Prepare solutions

Prepare 400 mL of buffer solution. Adjust the pH to 7.4 with NaOH.

1

	Concentration [mM]	Molar Mass [g/mol]	Mass [mg]
NaCl	134	58,44	3132,38
KCl	4,0	74,56	119,30
NaH2PO4	1,2	156,01	74,88
HEPES	10	238,30	953,20
MgSO4	0,5	120,40	24,08
D-Glucose	11	180,16	435,99

Buffer solution

2 Prepare 10 mL of 500 mM CaCl2 solution.

	Concentration [mM]	Molar Mass [g/mol]	Mass [g]
CaCl2	500	147,02	0,74

500 mM CaCl2 solution

3 Prepare 50 mL of 100 mM CaCl2 solution.

	Concentration [mM]	Molar Mass [g/mol]	Mass [g]
CaCl2	100	147,02	0,74

100 mM CaCl2 solution

4 Separate 200 mL of buffer solution and add 0,5 mL of 500 mM $CaCl_2$ solution to obtain a buffer solution with 1,25 mM of Ca^{2+}.

5 Separate 50 mL of buffer solution and add 25 µL of 100 mM $CaCl_2$ solution to obtain a buffer solution with 50 µM of Ca^{2+}.

6 Separate 15 mL of buffer solution and add 15 µL of 100 mM $CaCl_2$ solution to obtain a buffer solution with 100 µM of Ca^{2+}.

7 Separate 15 mL of buffer solution and add 30 µL of 100 mM $CaCl_2$ solution to obtain a buffer solution with 200 µM of

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Ca^{2+}.

8 Separate 15 mL of buffer solution and add 75 µL of 100 mM $CaCl_2$ solution to obtain a buffer solution with 500 µM of Ca^{2+}.

9 Separate 15 mL of buffer solution and add 150 µL of 100 mM $CaCl_2$ solution to obtain a buffer solution with 1 mM of Ca^{2+}.

On the day of the experiment

10 Separate 100 mL of buffer solution with 1,25 mM of Ca^{2+}, oxygenate the solution and keep it in the fridge at 4 °C.

11 Heat and maintain the remaining 100 mL of buffer solution with 1,25 mM of Ca^{2+} at 37 °C.

12 Prepare the digestion buffer. Add 10 mg of type II collagenase in 40 mL buffer solution with 20 µM of Ca^{2+}. Maintain the digestion buffer in the fridge at 4 °C.

13 Heat and maintain the buffers solutions with different Ca^{2+} concentrations (50 µM, 200 µM, 500 µM, 1 mM) at 37 °C.

Experimental setup

14 Assemble a constant flow Langendorff apparatus using a peristaltic pump, tubing, cannula and a heat exchanger coil. Connect the exchanger coil to a circulating water bath temperature set at 37 °C.

15 Connect a thermometer to the outflow of the Langendorff apparatus, to measure the temperature of the heated solution.

16 Fill the Langendorff apparatus with pre-heated buffer solution with 1,25 mM of Ca^{2+}. Eliminate possible air bubbles. Adjust the peristaltic pump to a flow rate of 5,5 mL/min at outflow from the cannula.

Myocyte isolation

17 Inject mouse with heparin solution (5000 U/Kg), via intraperitoneal injection.

18 After 30 min, anesthetize rat with a single intraperitoneal injection of sodium thiopental (50 mg/kg). When fully anesthetized (no response to strong foot pinch), euthanize the animal by cervical dislocation.

19 Open the chest and identify the aorta. Excise the heart, being careful not to damage the aorta. Place the heart in 4 °C, pre-oxygenated buffer solution with 1,25 mM Ca^{2+}.

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20 Cannulate the heart by the aorta in the cannula attached to the Langendorff system. Secure the aorta to the cannula by tying a loop of silk suture.

21 Perfuse the heart with a buffer solution with 1,25 mM Ca^{2+} at a flow of 5,5 ml/min for 10 min.

22 Switch the solution to Ca^{2+} free buffer and perfuse the heart with this solution at a flow of 7,5 ml/min for 5 min.

23 Switch the solution to digestion buffer and perfuse the heart with this solution at a flow of 7,5 ml/min for 5 min. Recirculate the solution with the enzyme. Keep the perfusion until the heart become flaccid and pale (20 – 30 minutes approximately).

24 Remove heart from cannula and place in petri dish with buffer solution with 50 µM Ca^{2+}. Cut the heart into small chunks and pipette several times with a transfer pipette to further disperse cells.

25 Filter the cell suspension into two 15 ml conical tube through a 250 µm nylon mesh filter.

26 Centrifuge the cell suspension for 3 minutes, 35g.

27 Aspirate the supernatant, and resuspend the pellet to buffer solution with 100 µM Ca^{2+}.

28 Allow cells to pellet for 10 min. Aspirate the supernatant, and resuspend the pellet to buffer solution with 200 µM Ca^{2+}.

29 Repeat last step, until cells are in the buffer solution with 1 mM Ca^{2+}.

Measuring contractility

30 Add some drops of buffer solution with cells to C-Stim chamber (IonOptix, USA).

31 Adjust syringe pump to perfuse (1 ml/min) buffer solution with 1 mM Ca^{2+} into the chamber. Adjust the temperature controller to 37 °C to pre-heat the solution in the chamber inlet and attach vacuum aspiration to chamber outlet.

32 Use 40X objective to locate desired cell. A healthy cell should be rod shaped and not spontaneously contracting.

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33 Set the electrical stimulator for bipolar pulses with 5 ms pulse duration, 25 V pulse amplitude and 1 Hz frequency.

34 Adjust camera rotation so cell is aligned horizontally on the screen.

35 In the software Ionwizard (IonOptix, USA), align edge detection bars to each end of myocyte and adjust threshold for contractility measurement with myocyte length variation.

36 Turn the electrical stimulator on and pace cells. Record tracing for posterior analysis.

37 Still in the software, align sarcomere detection on an area of cell with uniform sarcomeres. Choose a longer bar in the longitudinal axis to include as many sarcomeres as possible and improve contractility measurement with sarcomere length variation.

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