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# Preparation of Adult Rat Ventricular Myocytes for FRET Imaging Experiments

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ABSTRACT

This is a protocol describing method for isolating rat cardiac myocytes for use in FRET-based imaging experiments.

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GUIDELINES

The protocol is in compliance with the Guide for the Care and Use of Laboratory Animals as adopted by National Institutes of Health and approved by the Institutional Animal Care and Use Committee at the University of Nevada, Reno.



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MATERIALS TEXT

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2,3-Butanedione monoxine **Sigma – Aldrich Catalog #31550**

Penicillin-Streptomycin (5,000 U/mL) **Thermo Fisher Catalog #15070063**

Insulin-Transferrin-Selenium-Ethanolamine (ITS -X) (100X) **Thermo Fisher Catalog #51500056**

collagenase type 2 **Worthington Biochemical Corporation Catalog #LS004179**

protease type XIV **Sigma Aldrich Catalog #P5147**

Minimum Essential Medium with Hanks salts and L-glutamine **Gibco - Thermo Fischer Catalog #11575-032**

1 Anesthetize adult Sprague-Dawley rat (250-300 mg) with intraperitoneal injection of pentobarbital (150 mg/kg).

2 After the animal has entered a deep plane of anesthesia (loss of corneal and toe pinch reflexes), remove the skin over the chest area. Open up the chest cavity using scissors to cut along both sides of the sternum. Remove the heart and place it in physiologic saline solution (PSS) containing (in mM) NaCl 140, KCl 5.4, $MgSO_4$ 2.5, $CaCl_2$ 1.5, glucose 11, and HEPES 5.5 (pH 7.4) at 37$^{o}$C .

3 Gently massage the heart by hand to expel excess blood from the ventricles. Perfuse the coronary arteries with Ca2+-containing PSS by inserting a cannula, attached to a syringe, into the aorta. Ensure that the coronaries are rinsed free of blood.

4 Attach the heart to a Langendorff apparatus, by cannulating the aorta, and perfuse with Ca2+ containing PSS at 37$^{o}$C. Maintain a flow rate of 5 ml/min for approximately 5 minutes using constant pressure.

5 Switch the solution perfusing the heart to $Ca^{2+}$-free PSS and continue for 5 minutes. Make sure that the heart stops contracting to ensure that all $Ca^{2+}$ containing solution has been rinsed from the heart.

6 Switch to a $Ca^{2+}$-free PSS containing collagenase type 2 (~1 mg/ml,) and protease type XIV (~0.1 mg/ml, Sigma-Aldrich) and continue to perfuse the heart for 6 to 7 minutes.

7 Remove the heart from the Langendorff apparatus, placing it in a dissecting dish with KB solution containing (in mM) $K^+$ glutamate 110, $KH_2PO_4$ 10, KCl 25, $MgSO_4$ 1.2, taurine 20, creatine 5, EGTA 0.5, glucose 20, HEPES 5 (pH 7.2).

8 Dissect the ventricles free from other tissue and cut into small pieces (approx. 2 mm x 2 mm). Transfer the pieces to a small beaker with 5 ml of fresh KB solution and mince with fine scissors. .

Gently triturate the minced tissue with a blunt Pasteur pipette and then filter through a 500 µm polyester mesh, adding



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9 fresh KB solution to the tissue as needed to facilitate release of additional cells. Collect the filtrate containing the isolated myocytes.

10 Allowed the myocytes collected in the filtrate to settle in the KB solution maintained at 37°C for 25 minutes. Then aspirate the supernatant, and resuspend the pellet of isolated cells in fresh KB solution, allowing the cells to settle one more time before culturing.

11 Aspirate the supernatant and resuspended the isolated cells in Minimum Essential Medium (MEM) containing bovine serum albumin (1 mg/ml), 2,3 butanedione monoxime (10 mM), insulin/transferrin/selenium (1X), and penicillin/streptomycin.

12 Transfer the resuspended cells to six-well plates and then place them in an incubator for approximately 1 hour.

13 After the cells have settled in the dishes, aspirate the supernatant and replace it with fresh media containing viruses expressing appropriate FRET-based biosensors. Return to cells to the incubator, monitoring for biosensor expression over the next 48 to 72 hours.



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