Apr 07, 2022

Isolation of Adult Pig Ventricular Myocytes

Robert Harvey[1], Shailesh Agarwal[1]

[1]University of Nevada, Reno

dx.doi.org/10.17504/protocols.io.ba8iihue

This protocol is for the isolation of adult pig ventricular myocytes.

DOI

dx.doi.org/10.17504/protocols.io.ba8iihue

Robert Harvey, Shailesh Agarwal 2022. Isolation of Adult Pig Ventricular Myocytes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ba8iihue

protocol ,

Jan 10, 2020

Apr 07, 2022

31722

This 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. All surgical procedures conducted by a licensed veterinarian.

MATERIALS

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

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

1 Sedate adult pig (25 to 50 kg) with Telezol (500 mg, intramuscular). Administer atropine (1 mg, subcutaneous), to control salivary and bronchial secretions, and (200 mg, intravenous), to facilitate tracheal intubation. Anesthetize the animal with isoflurane (2-4%, inhalation).

2 Shave the chest area and wash with betadine antiseptic solution. Check for loss of corneal and pain reflexes to ensure that the animal is in a deep anesthetic plane. Open the chest cavity by peeling back the skin over the left side of the chest wall and then making an incision between the 4th and 5th ribs. Use rib spreaders to hold open the incision.

3 Open the pericardium and remove the heart, placing it in ice cold cardioplegia solution containing (in mM) NaCl 130, KCl 27, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 50, HEPES 6 (pH 7.4). Rinse the heart free of blood and transport to the laboratory in cardioplegia solution on ice.

4 Dissect the heart, removing a section of the left ventricular free wall supplied by the left anterior descending (LAD) coronary artery.

5 Attache the ventricular free wall to a Langendorff apparatus by cannulating the proximal end of the LAD coronary artery, and perfuse the tissue with Ca^{2+}-free solution containing (in mM) NaCl 140, KCl 5.4, $MgCl_2$ 2.5, glucose 11, HEPES 5.5 (pH 7.4) maintained at 37^{o}C,.

6 Ligate any leaking arteries at the periphery of the free wall in order to maintain perfusion pressure throughout the bulk of the tissue. Continue perfusion using, constant pressure, until the effluent is free of any remaining blood (about 10 minutes).

7 Switch perfusion to Ca2+-free solution containing collagenase type 2 (~200 U/ml) and protease type XIV (~1 mg/ml) for 20 minutes.

8 Stop enzyme digestion of the tissue by perfusing with enzyme-free 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) for 2 minutes.

9 Remove the tissue from the Langendorff apparatus and separate into different regions as necessary. The top half can be used to obtain myocytes from the base of the free wall, while

Citation: Robert Harvey, Shailesh Agarwal Isolation of Adult Pig Ventricular Myocytes
https://dx.doi.org/10.17504/protocols.io.ba8iihue

the bottom half can be used to obtain myocytes from the apex.

10 Cut the tissue into small pieces, mince with scissors, and then filter through a 500 µm polyester mesh. Collect the filtrate containing isolated myocytes. Allow the cells to settle in KB solution maintained at 37°C for 25 minutes.

11 Aspirate the supernatant and then resuspend the pellet of isolated myocytes in Ca^{2+} containing solution for use in experiments.

Citation: Robert Harvey, Shailesh Agarwal Isolation of Adult Pig Ventricular Myocytes
https://dx.doi.org/10.17504/protocols.io.ba8iihue