Neonatal rat cardiomyocyte harvest protocol

Protocol for neonatal rat cariomyocyte isolation edited from a protocol kindly provided by:

Dr. Richard Pattern
Tufts-New England Medical Centre
Molecular Cardiology Research Centre
Boston, MA

Reagents:

Dissociation buffer
116 mM NaCl,
20 mM Hepes,
0.8 mM Na_2HPO_4,
5.6 mM glucose,
5.4 mM KCl,
0.8 mM $MgSO_4$,
pH 7.35,

Enzyme buffer
Dissociation buffer plus the following:
0.6 mg/ml of pancreatin
0.4 mg/ml Collagenase Type II

Notes / tips:
Pre-sterilize: Tissue Dissociation Bottles with lids and Instruments
Serial digestions are performed at 37°C;
Use 1 to 2-day old Sprague-Dawley Rat pups.

Procedure:

1. Pre-refrigerate the pups at 4°C for about 20 min. Use no more than two or three litters per prep as increasing the number of pups generally does not lead to a dramatic increase in yield.

2. Place in the hood: Sterile Instruments; 4-10 cm^2 tissue culture plates. One 100 ml beaker with 70% EtOH; One 500 ml beaker with glove lining for carcasses; One rectangular dish full of ice. Place 15 ml of dissociation buffer alone (no enzymes) into a 10cm^2 plate and keep solution chilled on the ice. Place 10 ml of enzyme solution into another 10 cm^2 plate and keep cool on ice.

The instruments should be placed in the other two 10cm^2 plates. Into the first: Scissors and small forceps. Into the second: micro-scissors, scalpel with size number 15 blade, and long forceps. Place sterile lids on these until use.

3. With the pups at your left side and using one pup at a time:

4. Decapitate first using large scissors.

5. Dip the body in 70% EtOH nearly completely (at least to the lower abdomen)

6. Make one cut with scissors to the left side of the sternum down to the diaphragm

7. Remove the heart with forceps and place in dissociation buffer (without enzyme) on ice.

Repeat the above for each pup.

8. After all pups have been euthanized and hearts removed, dispose of the carcasses (tie end of glove and place at –20°C) and remove tray on which the hearts were removed; then remove instruments and the two beakers used and place in or next to the sink to be cleaned later. Wipe down the inside of the hood with 70% EtOH.

9. Next, place the 10 cm dish containing the hearts onto a 2 cm^2 gauze piece, and using the long forceps and scalpel, cut the great vessels and atria away from the ventricles of each heart and place the ventricles into the enzyme solution on ice.

10. After all hearts have been trimmed, take the 10 cm^2 plate containing the hearts and enzyme solution, remove from ice and place onto 2 cm^2 gauze. Mince each heart holding the apex or base with the forceps and using the micro-scissors, cut the ventricles transversely and sagittally approximately 7-12 times per heart.

11. After all hearts have been minced, draw up suspension into a pipette (10 - 25 ml) and deposit the suspension into one of the sterilized tissue dissociation bottles. Place lid on the bottle, secure into the 37°C water bath, and shake at approximately 80-86 rpms.

12. Perform serial digestions for the following time periods:

First - 5 minutes, discard as most cells in the suspension are erythrocytes.
Second - 20 minutes
Third and fourth - 25 minutes each.
Fifth - 15 minutes
Sixth - 10 minutes.

If there are still large tissue fragments left after the fourth digestion, the time can be extended for the 5th and 6th digestions by 5-10 minutes.

13. After each digestion, the cell suspension is placed immediately in 2 ml of neonatal calf serum that is then centrifuged at 660 rpm (about 100 G) for 5 minutes. The resultant pellet is then resuspended in 4 ml of NCS and kept at 37°C in the incubator.

14. After all digestions, the cell-suspensions in NCS are pooled and centrifuged again for 5 minutes at 660 rpm. The pellet is then re-suspended in Plating media (either Ham's F10 with 10% horse serum and 5% charcoal-stripped fetal bovine serum (FBS) containing 100 µM bromo-deoxyuridine, 100 µg/L penicillin and streptomycin or the same additives except in DMEM with 7% charcoal stripped bovine growth serum).

15. The suspension is passed through a 70 µm nylon filter and pre plated for 75 minutes on 60 mm^2 plates (e.g use 4 plates, 4 ml of media each for two litters of rats).

16. After pre-plating, remove the cardiomyocyte-enriched fraction, from each and place fresh media on each and label as non-myocytes. Bring the total volume of the cell suspension to 10 ml/litter. Gently mix the cell suspension

and remove an aliquot of cells (usually 40µl) and place in 120 µl of trypan blue solution (1/4 dilution). Count cells in a hemocytometer and calculate the total yield using the following formula:

Average number of cells per 1mm block x 4 (dilution factor) x 10^4 = number of cells per ml of media.
Multiply this number by the total volume (usually 20ml/two litters) and this is your yield.

For plating onto 10 cm^2 plates: plate 4-8 million per plate. These need either to be plates pre-coated with gelatin: 1% gelatin in PBS place on the plate (usually only 1-2 ml is needed) for about 1-2 minutes, aspirated off, and the plate is air dried for 60 minutes. Or commercially available pre coated plates are available.

For plating onto p60s: Plate 1-1.3 million cells per plate.

For 6 well plates: 750K per well.

For 12 well plates: 200K per well.

For 24 well plates: 100K per well (I usually plate a total of 2.5-3 million cells per pate).

For 8 well chamber slides: 250-300K per whole slide or approximately 30-40K per well.

Generally, virus or transfection reagents (or both) should be added at the time of plating. Virus, and/or transfection reagents are removed on the following day when cells are serum deprived.