Adult Mouse Cardiac Myocyte Culture Procedure Protocol

(Liao Lab Protocol)

Prepare buffer:

1.Perfuse buffer: Ca^{++} free Tyrode buffer + 10mM Glucose + 10mM BDM (Sigma, B-0753) + 5mM Taurine (Sigma, T-0625) (PH=7.4@RT), filter it.
2.Transfer buffer:

Buffer A. perfuse buffer + BSA(5mg/ml, Sigma, catalog# A-7030) (PH=7.4@RT), needed to filter the buffer with 0.2 um filter

Buffer b. 1.2mM Ca^{++} Tyrode buffer + 5.5mM Glucose(PH=7.4@RT), needed to filter the buffer with 0.2 um filter

transfer buffer	buffer a	buffer b	total volume
0.06mM Ca++	9.5ml	0.5ml	10ml
0.24mM Ca++	8ml	2ml	10ml
0.6mM Ca++	5ml	5ml	10ml
1.2mM Ca++	0ml	10ml	10ml

Prepare culture medium:

1. Myocyte Plating Medium: MEM (GIBCO, catalog# 11575-032) +penciline(100U/ml) + 2mM L-Glutamine + 5% FCS+ 10mM BDM(Sigma, catalog# B-0753).
2. Short term myocyte culture medium: MEM (GIBCO, catalog# 11575-032) + pencilin(100U/ml) + 2mM L-Glutamine + BSA(0.1mg/ml, Sigma, catalog# A-7030).

3. Long term myocyte culture medium: MEM (GIBCO, catalog# 11575-032) + pencilin(100U/ml) + 2mM L-Glutamine + BSA(0.1mg/ml, Sigma, catalog# A-7030) +10mMBDM +10ug/ml Insulin + 5.5ug/ml Transferrin + 5ng/ml Selenium(100-fold dilution of the ITS supplement, Sigma, I-1884).

Put plating medium and culture medium into 2% CO_2 culture incubator (37° C) 2 hours before using.

Prepare Enzyme solution:

Collagenase D 0.3mg/g BW (Roche, Catalog#1-088-882)
Collagenase B 0.4mg/g BW (Roche, Catalog#1-088-823)
Protenase XIV 0.05mg/g BW (Sigma, Catalog# P-5147)
Dissolve all the Enzyme with 25ml perfuse buffer and filter.

Procedure:

Prepare the perfusion apparatus:

1. Set the circulating water bath so that the outflow from the tip of the cannula is 37oC when the perfuse flow is 3ml/ml.
2. Run 70% alcohol 15min through the perfusion system, then run 100ml of sterile water, then run perfusion buffer through the perfusion system 5min, eliminate air bubbles.
3. put all the surgical tools into 70% alcohol for 20min.

Coating Laminin: dissolve laminin(inventor Catalog#23017-015) with 1x PBS(GIBCO, Catalog#10010-023, (PH=7.4@RT)),10ug/mlPBS,coating (2ml per P60, 1ml per P35) culture dish prior to cell isolation.

Cell isolation:

1. Inject the mouse i.p. with 0.2cc heparin(1000IU/ml).
2. 10min later, i.p. with pentababital 50mg/kg BW, after the mouse is fully anesthetized, wipe the chest with 70% ethanol, open chest and take the heart out.
3. Put the heart in cold(4^0C) sterile saline, cannulate the heart and tie the aorta to the cannula with 5-0 silk thread.
4. Perfuse the heart with Ca^{++} free perfuse buffer for 3min at 3ml/min, Switch to enzyme buffer and perfuse for 7-10min at 3ml/min (enzyme buffer can recycled).
4. Once enzyme digestion of the heart is complete, cut the heart (only keep left and right ventricles) and put the heart into sterile P60 culture dish with transfer buffer a, separate the heart into small pieces gently with forceps, pipette several times with a sterile plastic transfer pipette. Filter the cell suspension into a 50cc centrifuge tube with 250um filter.15mins gravity later, transfer the cell to 0.06mM, 0.24mM, 0.6mM and 1.2mMCa^{++} transfer buffer step by step every 10mins.

Plating Myocyte and Culture:

Transfer myocyte from 1.2 Ca^{++}-Tyrode buffer to plating medium (the volume depend on the size and amount of culture dish (3ml for P60, 2ml for P35). Make sure the myocytes are resuspended well by gently pipetting and divided into the culture dish evenly. Place finished culture dish immediately in a 2% CO_2 incubator at 37° C. incubate for 1 hour to allow myocyte attachment.

After 1 hour, aspirate the plating medium and wash each dish with 1.5ml culture medium to remove unattached myocytes and debris and aspirate the wash. Add short term myocyte culture medium (2ml for P35, 3ml for P60), immediately return myocyte to the incubator.

Incubate the myocytes at 37° C in 2% CO_2 until use.

Long Term Myocyte Culture:

All the procedure is same as short term myocyte culture except switch short term myocyte culture medium to long term myocyte culture medium.