

Isolate adult cardiomyocytes using Langendorff Mode
(Liao Lab Protocol)

Male adult Wistar rats (250-350 g)

1. The rats are sedated by i.p. sodium pentobarbital injection
2. I.v. heparin 200 U in femoral vein (important that i.v., shortens digestion time by ~ 50% compared to heparin i.p.)
3. Excision of heart and storage (max. 1-2 min.) in cold (4° C) cardioplegic solution (KB: in mM KOH 85, KCl 30 KH₂PO₄ 30, MgSO₄ 3, EGTA 0.5, Hepes 10, l-glutamic acid 50, taurine 20, l-glucose 10, pH 7.4)¹
4. Cannulation of aorta and retrograde perfusion of the heart at a perfusion pressure of 90 cm H₂O (Gravity-System) with
 - a) non-recirculating Tyrode solution (in mM NaCl 137, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, Hepes 10, l-glucose 10, pH 7.4) for 5 minutes at 37° C to wash out blood
 - required flow ~ 120 – 180 drops per minute, unpaced HR 250-300
 - b) non-recirculating, nominally Ca²⁺-free Tyrode solution (in mM NaCl 135, KCl 4, MgCl₂ 1, NaH₂PO₄ 0.33, Hepes 10, l-glucose 10, pH 7.4) for 5 minutes at 37° C to loosen tight junction
 - required flow ~ 140-180 drops in first 30", > 100 drops per minute in 5. Min.
 - c) recirculating nominally Ca²⁺-free Tyrode solution (as b) containing 0.08% collagenase (Collagenase A, Boehringer Mannheim, Indianapolis, MN) and 0.02% protease XIV (P5147, Sigma Chemical Co., St. Louis, MO)
 - Timing is critical to ensure good quality of isolated cells – useful tips, stop after one min of steady flow, usually after 6 – 10 min, typically 8 min. The timing is highly depending on the batch of collagenase and heart weight.
 - d) KB solution (as above) 5 min. 37° C
5. Cut down heart, remove pericardium, trim off atria and RV

6. Slice LV in 2-3 mm pieces, agitate muscle pieces gently in KB solution (in small weight dish) at room temperature.
7. Filter loosened myocytes through 150 μm mesh
8. Store myocytes in KB for 60 min. at room temperature before use
9. Culture or transfer myocyte directly in Tyrode solution (as above, except glucose 5.5 and Ca^{2+} 1.2 mM)
10. In general: myocytes must be rectangular, sharp edges, no blebbs
Myocytes must stick spontaneously to cover glass and sustain flow of up to 2ml/min. If float away indication for over-digestion.

Collagenase: Collagenase A from Boehringer Mannheim

Presently: specific activity 0.2 u/mg

Potential contaminants: Trypsin 0.2 u/mg; Caseinase 691 u/mg;

Clostripain 2.9 u/mg

¹Isenberg G, Klockner U. Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium". *Pflugers Arch.* 1982;395:6-18