Isolation of Adult Mouse Cardiac Myocytes from Two or More Hearts
AfCS Procedure Protocol PP00000126
Version 1, 11/05/02

This procedure describes the isolation and culture of adult mouse cardiac myocytes from two or more hearts. This protocol is similar to the AfCS protocol PP00000125, Isolation of Adult Mouse Cardiac Myocytes from One Heart, but includes modifications for the digestion of two or more hearts in the same procedure and subsequent pooling of myocytes derived from the multiple hearts. The isolation procedure is performed by one or more technicians and routinely yields approximately 1 million rod-shaped myocytes per heart.

Procedure Setup
1. This procedure uses male C57BL/6 mice, 8 to 10 weeks old (20 to 30 g). Record strain, sex, and weight.
2. Prepare perfusion buffer; myocyte digestion buffer (MC digestion buffer); myocyte stopping buffers (MC stop 1 and MC stop 2); myocyte plating medium (MC plating medium); myocyte culture medium (MC culture medium); and laminin-coated dishes (see “Laminin Coating of Culture Dishes” below) fresh daily. Prepare 50 ml of MC digestion buffer for each heart, and store each 50 ml in a separate 50-ml Falcon tube. Perfusion buffer and MC digestion buffer should be warmed to 37 °C prior to use. Do not warm the enzyme for each heart until ready to isolate myocytes; exposure to prolonged high temperature may inactivate the enzyme. Equilbrate MC plating medium and MC culture medium at 37 °C in a 2% CO2 incubator (for at least 2 hr to adjust temperature and pH). Sterilize instruments in hot bead sterilizer.
3. Prepare the perfusion apparatus. Set the circulating water bath so that the outflow from the tip of the cannula is 37 °C. Check the flow rate of the pump and adjust to 3 ml/min. (Note: the temperature of the perfusate and the flow rate of the pump should be checked routinely. Over time, peristaltic pump tubing will fail and will need to be replaced every 2 to 3 months to maintain consistent flow rates. These routine checks are essential to maintain consistency in preparations). Note: two alternative systems for solution handling during perfusion are used (see “Perfusion Systems” below). The following instructions describe perfusion with system A. Differences for perfusion system B are noted below and detailed in the description of perfusion systems.
4. Run 100 ml of purified water through the perfusion system; then run perfusion buffer through the system for at least 5 min.
5. Add perfusion buffer and MC digestion buffer to the correct reservoirs, prime the perfusion system with buffers, eliminate air bubbles, and allow time to achieve temperature (about 15 min). Note: no prewarming is required for perfusion system B.
6. Add 10 ml of room temperature perfusion buffer to a 60-mm culture dish for heart collection. Add 20 ml of room temperature perfusion buffer to a 100-mm culture dish for heart cannulation, and place on an adjustable stage under the perfusion apparatus.
7. Position the cannula with the tip close to the surface of the perfusion buffer in the 100-mm dish. The pump can be set to a very slow rate (0.4 ml/min, for example) during the cannulation, but this is not necessary.

8. Cut several small pieces of 6-0 surgical silk (10 to 15 cm), knot loosely, and place on the adjustable stage (this will be used to secure the aorta to the cannula).

**Removal and Cannulation of the Heart**

Steps 9 through 22 may be performed on more than one heart at the same time, if parallel apparatuses and technical help are available. Points that can be used for initiation of parallel processing are indicated below in italics.

9. Inject a mouse i.p. with 0.5 cc heparin diluted in phosphate buffered saline (PBS) to 100 IU/ml.

10. Anesthetize the mouse with isoflurane and 100% O₂. Set the isoflurane atomizer dial to 3% (scale 1% to 5% of total flow), turn the O₂ valve to 0.5 L/min, and place animal inside the induction chamber. When the mouse is anesthetized, it will lose consciousness and roll over on its side. Check with a toe pinch to ensure that the mouse is fully anesthetized. Transfer the mouse to the surgery/perfusion area and place under a nose cone connected to the anesthesia system. *If parallel digestion of hearts is planned, two or more mice can be anesthetized at the same time.*

11. Once the animal is on the surgery area, wipe the chest with 70% ethanol and adjust the isoflurane (usually 1.5%) as necessary to ensure proper level of anesthesia (movement indicates that the anesthesia is too shallow, whereas irregular respiration indicates that it is too deep). Check with a toe pinch to ensure that the mouse is fully anesthetized.

12. Open the peritoneal cavity and chest with small scissors and use forceps to peel back the rib cage to expose the heart. Lift the heart gently using forceps. Identify and cut the pulmonary vessels, which will make it easier to identify and cut the aorta. Cut the aorta at about 2 mm from its entry into the heart and immediately place the heart in a 60-mm dish containing 10 ml of perfusion buffer at room temperature. Too long a section of aorta will make the aorta harder to identify and lift onto the cannula. Conversely, too short a section of aorta will make it harder to tie off the aorta on the cannula and increase the likelihood of pushing the cannula through the aortic valve, preventing good perfusion.

13. Remove extraneous tissues (thymus and lungs), if necessary, and transfer heart to the 100-mm dish with perfusion buffer at room temperature.

14. Cannulate the heart using fine-tip forceps to slide the aorta onto the cannula so that the tip of the cannula is just above the aortic valve (check the 1-mm notch on the cannula to ensure proper cannulation; see description of cannula below). Attach a small brass clip at the end of the cannula to prevent the heart from falling. Start the perfusion immediately (3 ml/min). Tie the aorta to the cannula with 6-0 silk thread. Total time to cannulate the heart should be less than 1 min. *Note: using magnifying lenses or a dissecting microscope will make the heart and aorta easier to visualize and cannulate.*
Heart Perfusion and Enzyme Digestion
15. Perfuse the heart with perfusion buffer for 4 min at 3 ml/min (this flushes blood from the vasculature and removes extracellular calcium to stop contractions). Measure the temperature of the heart with an insulated wire probe attached to a digital thermometer, placing temperature probe into ventricle to ensure temperature is 37 °C. (Note: this does not need to be done each time, but should be done periodically to ensure reproducibility).

16. Switch to MC digestion buffer and perfuse for 8 to 10 min at 3 ml/min (digestion times can vary from heart to heart). If the heart is well perfused during the enzyme digestion, the heart will become swollen and turn slightly pale, and separation of muscle fibers on the surface of the heart may become apparent. Note: during perfusion of one heart, a second heart can be hung for digestion on a parallel apparatus following steps 10 through 14 or 11 through 14, if the second mouse is already anesthetized. Dissociation of myocytes from the two hearts (see below) can be interspersed.

Myocyte Dissociation
17. Once enzyme digestion of a heart is complete (heart appears swollen, pale, and flaccid), cut the heart from the cannula just below the atria using sterile, fine scissors. Place the ventricles in a 60-mm dish containing 2.5 ml of MC digestion buffer. From this point forward, sterile techniques should be maintained, and all subsequent steps are performed under a laminar flow culture hood.

18. Cut the heart in half and begin to gently tease the ventricles into several small pieces with fine forceps. Pipette gently several times with a sterile plastic transfer pipette (2-mm opening). This process takes 60 to 90 sec. (Note: the tissue should be very flaccid, almost falling apart on its own, and require very little force to pull apart, which will indicate a good digestion.)

19. Transfer the cell suspension to a 15-ml polypropylene conical tube. Rinse the plate with 2.5 ml of room temperature myocyte stopping buffer 1 (MC stop 1), and combine with the cell suspension for a final volume of 5 ml. (Note: MC stop 1 contains serum to inactivate proteases; the final calf serum concentration is 5%, and the final calcium concentration is 12.5 µM).

20. Continue to dissociate the heart tissue gently, using sterile plastic transfer pipettes with different sized openings (2-mm, 1.5-mm, and then 1-mm diameters), until all the large pieces of heart tissue are dispersed in the cell suspension. Avoid vigorous agitation to minimize shearing of the cells. This process should take 3 to 5 min.

21. Count rod-shaped and round myocytes using a hemacytometer (see "Counting Myocytes with a Hemacytometer" below). Calculate the total number of myocytes, the number of rod-shaped myocytes, and the percent of rod-shaped myocytes. Record these values as the initial number of cells obtained. (Note: the important number for comparison between labs is the total number of rod-shaped myocytes, as the method for counting the number of round myocytes is often hard to standardize. However, the percent of rod-shaped myocytes is still a useful number as it can reflect the quality of the isolation. If at this point, the total cell
yield from a specific heart is low [less than 1 million], or the percent of rod-shaped myocytes is low [less than 60%], the cells prepared from this heart should be discarded.)

22. **At this time one can begin preparation for digestion of the next heart on the same apparatus.** Replenish perfusion buffer and MC digestion buffer in the appropriate reservoirs. Anesthetize the next mouse (refer to step 9). Remove and hang the next heart, then proceed to step 23 with the previous heart while the next heart is being perfused (steps 10 through 16). **Alternately, if multiple hearts were digested at the same time, proceed to step 23 to pool and continue the preparations of cells that pass the quality test in step 21.**

**Calcium Reintroduction**

(All steps are conducted at room temperature.)

23. Allow the myocytes to sediment by gravity for 8 to 10 min in the 15-ml tube(s) while counting the myocytes. Transfer the supernatant to a new 15-ml tube and centrifuge for 1 min at 180 x g.

24. Resuspend the new pellet in 5 ml of room temperature myocyte stopping buffer 2 (MC stop 2), combine with the original sedimented myocytes, and adjust to a total volume of 10 ml with MC stop 2. (Note: MC stop 2 contains 5% serum, and the final calcium concentration is 12.5 µM). If multiple hearts have been digested at the same time, combine in pairs by suspending the settled cells from one heart in 2.5 ml of MC stop 2 and transferring onto the settled cells from a second heart. **Suspend the pellets from the supernatants of each of the two hearts in 5 ml of MC stop 2 and add to the settled cells. Adjust to a total volume of 10 ml with MC stop 2 and proceed as for single hearts below.**

25. Transfer the combined pellets (in 10 ml) to a 60-mm nonstick Valmark dish. To evenly distribute the myocytes in the dish, move the dish forward and backward and side to side, but do not swirl the dish. (Note: the myocytes in the 60-mm Valmark dish can be monitored under a microscope during calcium reintroduction. If during the reintroduction period, the myocytes begin to round up, or become very granular, the myocyte quality might be poor. A decision may be made as to whether to continue with the prep or to start over, depending on the judgment of the technician.)

26. Add 50 µl of calcium chloride, 10 mM (10 mM CaCl₂); final concentration is increased to 62 µM. Mix well and incubate for 4 min at room temperature.

27. Add 50 µl of 10 mM CaCl₂; final concentration is increased to 112 µM. Mix well and incubate for 4 min at room temperature.

28. Add 100 µl of 10 mM CaCl₂; final concentration is increased to 212 µM. Mix well and incubate for 4 min at room temperature.

29. Add 30 µl of calcium chloride, 100 mM (100 mM CaCl₂); final concentration is increased to approximately 500 µM. Mix well and incubate for 4 min at room temperature.

30. Add 50 µl of 100 mM CaCl₂; final concentration is increased to approximately 1 mM. Mix well and incubate for 4 min at room temperature.
31. Transfer the myocytes to a new 15-ml tube and leave at room temperature while any successive hearts are prepared. After all of the hearts are prepared, continue with step 32.

32. Transfer the supernatant from each tube to another new 15-ml tube and centrifuge for 1 min at 180 x g.

33. Resuspend and combine both pellets from each sample in 5 ml of MC plating medium (1.2 mM Ca\textsuperscript{2+}) at 37 °C.

34. Count rod-shaped and round myocytes using a hemacytometer (four counts, or both sides of two separate hemacytometers). Calculate the total number of myocytes, the number of rod-shaped myocytes, and the percent of rod-shaped myocytes. **Record these values as the number of myocytes for plating.** The most important number is the **number of rod-shaped myocytes.** If the myocyte number is low or myocyte quality is poor, a decision may be made as to whether to continue with the prep or to start over, depending on the judgment of the technician.

**Plating Myocytes, Myocyte Attachment, and Culture**

35. Calculate the total rods in the preparations to be pooled, and determine the volume required to adjust the concentration of rod-shaped myocytes to 25,000 rod-shaped myocytes/ml. Combine all of the preparations in a 50-ml Falcon tube and adjust the volume with MC plating medium (if the volume is greater than 50 ml, additional 50-ml tubes can be used, with myocytes divided evenly between tubes, or the myocytes may be combined into a 250-ml centrifuge tube). MC plating medium should have been equilibrated for 2 to 3 hr at 37 °C in a 2% CO\textsubscript{2} incubator. Make sure the myocytes are resuspended well by gently pipetting (use a 10-ml pipette). (Note: at this stage the myocytes are in MC plating medium, which contains butanedione monoxime [BDM], a contraction inhibitor [also found in the perfusion buffer], and 5% calf serum.)

36. Set up trays to plate no more than 20 dishes per tray at a time (this avoids pH changes as medium is exposed to air). Plate the appropriate amount of rod-shaped myocytes in desired vessels: 2 ml (containing 50,000 rod-shaped myocytes) in a laminin-coated, 35-mm dish; 6 ml (containing 150,000 rods) in a 60-mm laminin-coated dish (see “Laminin Coating of Culture Dishes” below). This is a density of roughly 52 rod-shaped myocytes per mm\textsuperscript{2}. Use a 5-ml pipette to plate two 35-mm dishes at once to prevent myocytes from settling in the pipette. Use a 10-ml pipette to plate single 60-mm dishes. During the plating procedure, resuspend the myocytes constantly to ensure they do not settle to the bottom of the tube, which will cause variation in plating density. Once the myocytes are plated, mix the myocytes in the dishes by gently sliding the tray forward and backward and side to side 3 to 4 times on the surface of the culture hood (in a cross-like pattern); never swirl the medium in the dish or the myocytes will not plate evenly. Place finished trays immediately in a 2% CO\textsubscript{2} incubator at 37 °C. Incubate for 1 hr to allow myocyte attachment.

37. After 1 hr, aspirate the plating medium with a sterile Pasteur pipette into a vacuum flask. Wash each dish with approximately 1.5 ml MC culture medium to remove unattached myocytes and debris and aspirate the wash. (Note: when changing
the medium, do not process more than 20 dishes [1 tray] at once. Once myocytes are attached, always add medium gently to the side of a dish.)

38. Add MC culture medium, which has been equilibrated at 37 °C in a 2% CO₂ incubator for at least 2 to 3 hr, to the washed cells (1 ml medium to 35-mm dishes; 3 ml medium to 60-mm dishes). Immediately return myocytes to the incubator. (Note: at this stage, the myocytes are in MC culture medium, which contains 0.1 mg/ml bovine serum albumin [BSA], but no BDM or calf serum.)

39. After an additional hour, count the number of rod-shaped myocytes and round myocytes. Count myocytes in three different dishes (from early, middle, and late in plating). Count at least four random fields using the 20x objective. Average the count of the four fields for each dish, and then average the three dishes. Calculate the total number of myocytes, the number of rod-shaped myocytes, and the percent of rod-shaped myocytes. For example,

\[
\text{Total myocytes} = \frac{\text{no. of myocytes counted}}{\text{no. of fields}} \times \text{area factor}
\]

(The area factor needs to be empirically determined for each microscope lens being used. Either total myocytes/cm² or total myocytes/dish are calculated; the latter allows determination of total myocytes in the preparation based on the number of dishes plated.)

\[
\text{Total rod-shaped} = \frac{\text{no. of rods counted}}{\text{no. of fields}} \times \text{area factor}
\]

\[
\% \text{ Rod-shaped} = \frac{\text{no. of rods}}{\text{total number of cells}}
\]

Record these values as the number of myocytes at T0.

40. Incubate the myocytes at 37 °C in 2% CO₂ until use. (Note: MC culture medium has Hanks’ Balanced Salt Solution with 0.35 g/L of NaHCO₃, giving a pH of 6.9 to 7.0 in a 2% CO₂ incubator.)

41. Count again at 24 hr or before use (18 to 24 hr).

Cleaning the Perfusion Rig

42. Clean the perfusion system by filling the reservoirs with purified water and rinsing the system. Next, fill the reservoirs and tubing with 70% ethanol and let the tubing soak in ethanol for at least 20 min. Lastly, run the ethanol through and wash the system twice with purified water.

Laminin Coating of Culture Dishes

1. Thaw laminin stock solution (laminin stock) on ice prior to coating the plate.
2. Add 10 ml of ice-cold phosphate buffered saline (PBS), 1X (CaCl₂/MgCl₂-free) to the laminin stock for a final concentration of 10 µg/ml.
3. Add 1.5 ml of laminin coating solution to cover the bottom of 35-mm plates, and incubate at 37 °C for 2 hr. Use proportionately more solution to coat 60-mm plates.
4. Remove laminin coating solution just prior to plating the myocytes.
5. Use the plates on the day they are prepared.

Counting Myocytes with a Hemacytometer

1. Mix myocytes well and use a 20-µl pipette to take a 10-µl sample.
2. Load both sides of the hemacytometer.
3. Count both sides, counting the four 1-mm corner squares and the 1-mm center square on each side (10 squares total). When counting, include myocytes that are on the top and left-side lines, but not the bottom and right-side lines.

4. Count both rod and round myocytes.

5. To determine cell number, multiply the total number of cells by 1,000 to get the myocytes per ml.

**Reagents and Materials**

Mice (8- to 10-week-old males): Charles River Laboratories; catalog no. C57BL/6

Perfusion buffer: AfCS Solution Protocol PS00000451

Myocyte digestion buffer (MC digestion buffer): AfCS Solution Protocol PS00000447

Myocyte stopping buffer 1 (MC stop 1): AfCS Solution Protocol PS00000449

Myocyte stopping buffer 2 (MC stop 2): AfCS Solution Protocol PS00000450

Myocyte plating medium (MC plating medium): AfCS Solution Protocol PS00000448

Myocyte culture medium (MC culture medium): AfCS Solution Protocol PS00000446

Falcon tube, 50 ml: Fisher Scientific; catalog no. 14-959-49A

Incubator, Forma Scientific model 3110 series II, water-jacketed CO₂ incubator: Forma Scientific; model 3110. Or NAPCO model 6101F-0, microprocessor controlled automatic water-jacketed CO₂ incubator: NAPCO; catalog no. 51201068

[Note: see "Incubators" below for more information.]

Fyrite CO₂ monitor: Fisher Scientific; catalog no. 10-884-1

Hot bead sterilizer: Fine Science Tools; catalog no. 18000-45

Perfusion apparatus: see "Perfusion Systems" below

Cannula: see "Cannula" below

Falcon culture dish, 60 x 15 mm: Fisher Scientific; catalog no. 08-772-4C

Falcon culture dish (polystyrene), 100 x 20 mm: Fisher Scientific; catalog no. 08-772-4F

6-0 silk: Fine Science Tools; catalog no. 18020-60

Heparin: AfCS Solution Protocol PS00000442

Isoflurane, 100 ml: Burns Veterinary Supply, Inc.; catalog no. 300-1532
100% O₂: local suppliers of standard gases for each laboratory

Isoflurane atomizer: VetEquip, Inc.; catalog no. 911103

Induction chamber: VetEquip, Inc.; catalog no. 941444

Ethanol, 70%: AfCS Solution Protocol ID PS00000011

Scissors: Fine Science Tools; catalog no. 14320-10

Forceps: Fine Science Tools; catalog no. 11052-10

Fine-tip forceps: Fine Science Tools; catalog no. 11251-15

Small brass clips: Radio Shack

Magnifying lenses: Cole-Parmer; catalog no. U-03886-00

Insulated wire probe: Cole-Parmer; catalog no. U-08506-75

Digi-Sense thermometer: Cole-Parmer; catalog no. 91100-20

Plastic transfer pipette, 2 mm: Fisher Scientific; catalog no. 13-711-7

Falcon polypropylene conical tube, 15 ml: Fisher Scientific; catalog no. 14-959-70C

Plastic transfer pipette, 1.5 mm: Fisher Scientific; catalog no. 13-711-27

Plastic transfer pipette, 1 mm: Fisher Scientific; catalog no. 13-711-9C

Hemacytometer: VWR International; catalog no. 15170-170

Nonstick Valmark dish, 60 mm: Midwest Scientific; catalog no. 901

Calcium chloride, 10 mM (10 mM CaCl₂): AfCS Solution Protocol PS00000440

Calcium chloride, 100 mM (100 mM CaCl₂): AfCS Solution Protocol PS00000441

Centrifuge tube, 250 ml: Corning Inc.; catalog no. 430776

Pipette, 10 ml: Fisher Scientific; catalog no. 13-675-20

Falcon culture dish (polystyrene), 35 x 10 mm: Fisher Scientific; catalog no. 08-772-4A

Pipette, 5 ml: Fisher Scientific; catalog no. 13-675-22
Pasteur pipette: Fisher Scientific; catalog no. 13-678-20A


Laminin stock solution (laminin stock): AfCS Solution Protocol PS00000444

Phosphate buffered saline (PBS), 1X (CaCl₂/MgCl₂-free): GIBCO/Invitrogen; catalog no. 10010023

Laminin coating solution: AfCS Solution Protocol PS00000443

**Cannula**

The cannula is a very important part of the prep. It is a 20-g needle with the sharp tip cut off and the nub filed flat and smooth (this is important to prevent snagging the aorta when hanging). It is also useful to make 1-mm and 2-mm notches on the bottom of the cannula to determine how far the cannula is inside the aorta.

**Incubators**

This protocol uses a Forma Scientific model 3110 series II water-jacketed CO₂ incubator or a NAPCO model 6101F-0 microprocessor controlled automatic water-jacketed CO₂ incubator. Other similar incubators may also be used. Read manual for precise operating instructions.

**In general**

1. Incubators should be set at 37 °C and 2% CO₂ (double-check with a Fyrite monitor, and follow the manufacturer’s instructions), and the water tray on the floor of the incubator should be full to maintain humidity. When setting the incubator, allow 24 hr to equilibrate before using.
2. When using the incubator, be aware that opening and closing the incubator will disturb the internal environment (CO₂ and humidity). Therefore, opening of the incubator should be kept to a minimum. Furthermore, although the CO₂ reading of the internal environment may return to normal on the incubator monitor, the medium in the incubator will take longer to equilibrate.
3. The CO₂ should be measured with a Fyrite CO₂ monitor each week, and the water pan should be filled if low.
**Perfusion Systems**  
**Perfusion System A**  
Perfusion system A has two halves, one for the perfusion buffer and one for the digestion buffer. Each half contains a water-jacketed reservoir for warming solutions to 37 °C, from which fluid is pumped through a peristaltic pump, through a heat exchanger to maintain solution temperature, through a bubble trap, then finally out through the cannula (see “Cannula” above for more information). Fluid flow is switched by changing the pump head attachment and diverting flow from one side to the other at the three-way valve above the cannula. To maintain the perfusate a 37 °C, the water-jacketed circulation system is maintained at 42 °C (but this must be empirically determined). See Figures 1-3 for details.

**Figure 1**

*Fluid Flow*

The rig has 2 halves (mirror images), one for calcium-free perfusate, the other for collagenase. Flow is switched by changing the pump head and switching the three-way valve at the cannula.

**Flow Path**
1. Uptake in the reservoir
2. Through the pump
3. Into the condensor
4. Into the bubble trap
5. Cut through the cannula
Figure 2
Part Numbers

1. Water Jacketed Beaker
   Ace Glass Cat# 5340-03
2. Spiral Condensor
   Ace Glass Cat# 9270-04
3. Adaptor (for tubing)
   Outer Joint 14/20
   Ace Glass Cat# 9069-05
4. Adaptor (for tubing)
   Inner Joint 14/20
   Ace Glass Cat# 9069-05
5. Bubble Trap
   Radnotti Glass Cat# 120149
6. Ring Clamp
   Radnotti Cat# 120149-RC
7. Tygon Tubing (for perfusate)
   3/32"x5/32"
   Radnotti Glass Cat# 120157
8. Tygon Tubing (for water jackets)
   1/2"x3/8"
   Radnotti Glass Cat# 120156
9. Stopcock, 1-way male
   Cole Parmer 30600-01
10. Stopcock 3-way male
    Cole Parmer 30600-03
Figure 3

Part Numbers
1. Dynamax Peristaltic Pump, Rainin Instruments, Catalog# 7103-054
2. PVC Manifold Tubing (for the pump), Rainin Instruments, Catalog# 99-628
3. Circulating Water Bath, Haake, Model# BC10
4. Illuminator, Light Pipe and Lens, Cole Parmer, Catalog# 41722-10, 41720-65, and 09743-20
5. Thermocouple Thermometer and Probe, Cole Parmer, Catalog# U-91100-20 and U08506-75
6. 4-bar Lab Stand with Stabilizer Bar, Radnoti Glass, Catalog# 159951-4
Perfusion System B

Perfusion system B utilizes preheating of perfusion solutions just prior to entry into the hanging heart (see Figure 4). Perfusion solutions are made and kept in 50-ml tubes at room temperature until use. Changes in perfusion solutions are achieved by stopping the peristaltic pump and moving the inlet tube to the container with the next desired solution. The total volume of solution contained in the perfusion tubing (inlet to cannula) is 4 ml; this is taken into account for the volumes of perfusion given in the procedures.

Perfusion solutions are pumped through a heat exchange coil where the temperature is raised to 37 °C for passage into the heart via the cannula. In practical terms, the temperature of the circulation water bath is set to about 39 °C so that the actual temperature of the heart during the perfusion is 37 °C. For digestion of multiple hearts at the same time, a single water bath can be used to equilibrate multiple heat exchange coils that are linked to the water bath in parallel. All other components are replicated for each perfusion apparatus. A picture of the perfusion system is shown in Figure 5.
Figure 5

Perfusion System B Materials
Cannula: See “Cannula” above for more information

Peristaltic pump, P-1: Fisher Scientific; catalog no. NC9304953

Water bath, heating circulator DC10-B3: HAAKE; catalog no. 4261702

Water-jacketed heat exchange coil, glass: custom manufactured locally by Custom Scientific Glass (phone: 213-343-3688)

Adjustable stage: Fisher Scientific: catalog no. 14-673-20

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