APPLICATION NOTE AN-900L-01

Isolated Heart Preparation and Mounting

Langendorff System – Model 900L | Danish Myo Technology A/S

Document information	
Document number	AN-900L-01
Applies to	Langendorff System 900L, mouse and rat hearts
Revision	1.0
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Related documents	User Manual: Section 3.7, Chapter 5 (Heart Mounting); AN-900L-02 (buffers and reagents)

1 Purpose and Scope

This note describes two ways to prepare an isolated rodent heart and mount it on the aortic cannula of the 900L: quick excision in cold buffer (Method 1), and intubation with in-situ cannulation of the aorta (Method 2). It follows the DMT example technique. The procedure is terminal: the animal is euthanized under deep anesthesia when the heart is excised. The 900L side of mounting (lowering the heart, setting pressure and flow) is in Chapter 5 of the User Manual.

WARNING Animal procedures must be approved by your ethics committee and follow local regulations. Only trained personnel should perform them. Surgical instruments are sharp, 70% ethanol is flammable, and biological material is potentially infectious. Wear gloves and eye protection.

NOTE The techniques are examples and are not independently validated. Follow your laboratory's approved protocols.

2 Equipment

2.1 Instruments

Item	Notes
900L Dissection Kit	1 surgical forceps, 2 large hemostats/clamps, 1 small scissors, 2 blunt-ended fine curved forceps, 1 eye scissor (#100073)
Silk suture	DMT #100341
Dissection stereomicroscope (#400598)	Recommended for new users. It makes dissection and cannulation much easier. Contact your DMT sales representative.
Rodent ventilator (Method 2)	DMT offers a rodent ventilator for mice and rats. Contact your sales representative.

2.2 Aortic Cannulas

Mouse set (#100357)	Gauge	Rat set (#100358)	Gauge
0.6 mm	23	–	–
0.8 mm	21	–	–
1.0 mm	19	–	–
1.2 mm	18	1.2 mm	18
1.5 mm	17	1.5 mm	17
–	–	2.0 mm	14
–	–	2.5 mm	n.a.
–	–	3.0 mm	11

Choose the cannula that fits the aorta without forcing it.

3 Before You Start

- ☐ The 900L is ready for mounting: see the checklist in Section 5.1 of the User Manual.
- ☐ Perfusion buffer is prepared, gassed and warm. Cold buffer for the dissection is on ice (AN-900L-02).
- ☐ The mounting tube extension is fitted with the right-size aortic cannula, flushed with buffer, with no air bubbles in the line.
- ☐ Anesthetic and anticoagulant (optional) are ready, as specified in your approved protocol.

This note does not specify the anesthetic, the anticoagulant or their doses.

4 Choose a Method

	Method 1	Method 2
Technique	Quick excision, cardiac arrest in ice-cold buffer	Intubation and ventilation, then in-situ cannulation of the aorta
Extra equipment	Ice, small beaker	Rodent ventilator
Section	5	6

5 Method 1: Quick Excision in Cold Buffer

CAUTION Do not let air enter the aorta or the cannula. Air bubbles block the coronary vessels.

1. Place ice-cold buffer in a small beaker on ice.
2. Prepare the mounting tube extension kit with the right-size aortic cannula. Flush the tubing and cannula with buffer. Make sure there are no air bubbles in the line.
3. Anesthetize the animal. Check the depth of anesthesia with the pedal withdrawal reflex. The corneal reflex can be used as a supplement.
4. When the animal is deeply anesthetized, quickly open the chest, cut out the heart and lungs together and transfer them to the ice-cold buffer.
5. Move the heart to a second tray of ice-cold buffer. Trim away lungs, veins and other tissue until the aorta is clearly visible and easy to reach with the cannula. Keep the heart submerged in the ice-cold buffer at all times.
6. While the heart is submerged, cannulate the aorta and tie a double knot around the aorta and cannula. The suture must catch one of the three grooves in the cannula, and the cannula must not go too far into the aorta, because it can block the coronary arteries (Section 8).

7. Continue with Section 7.

> **NOTE** Work as fast as possible. The shorter the time from excision to the start of perfusion, the better the outcome. Up to 3–5 minutes is acceptable for both rat and mouse hearts.

6 Method 2: Intubation and In-Situ Cannulation

In Method 2 the animal is intubated and ventilated, so the heart keeps oxygen supply while you work. Without ventilation, the lungs collapse when the chest is opened and the animal suffocates slowly.

6.1 Intubate and Ventilate

1. Induce anesthesia. When the animal is adequately anesthetized, fix its legs to a plate with strings and pins (Figure 1).
2. Clean the chest with disinfectant, for example 70% ethanol.
3. Cut the skin above the sternum.
4. Expose the trachea with blunt-ended fine curved forceps (Figure 2).
5. Gently slide a silk suture underneath the trachea (Figure 3) and tie a loose knot.
6. Make a small horizontal incision in the trachea.
7. Insert the endotracheal tube (Figure 4).
8. Tie a double knot to fix the tube in the trachea (Figure 5).
9. Connect the tube to the rodent ventilator (Figure 6) and start the ventilator.
10. Ventilate with atmospheric air. Example settings: mouse 0.20 mL per breath at 120 breaths/min. Rat 2 mL per breath at 60 breaths/min.
11. Check that the chest rises on both sides.

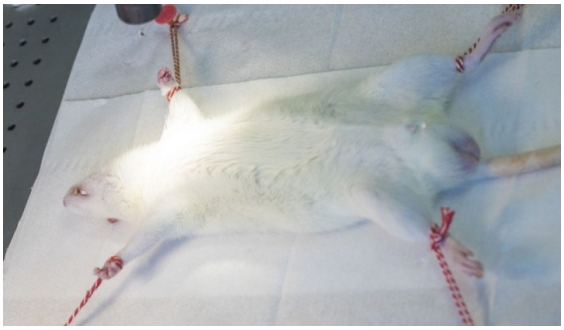

Figure 1 – Legs fixed to the plate.

Figure 2 – Trachea exposed.

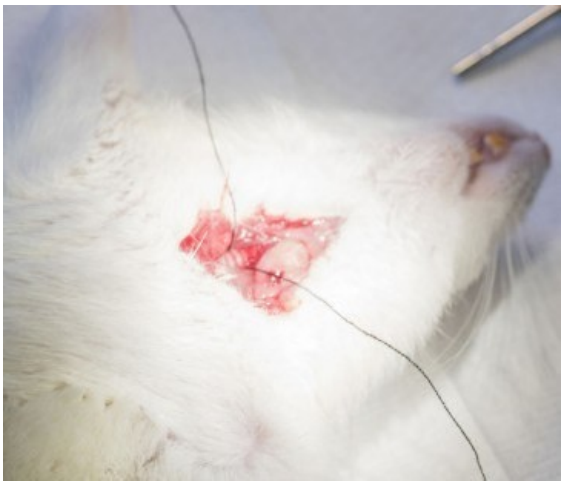

Figure 3 – Suture under the trachea.

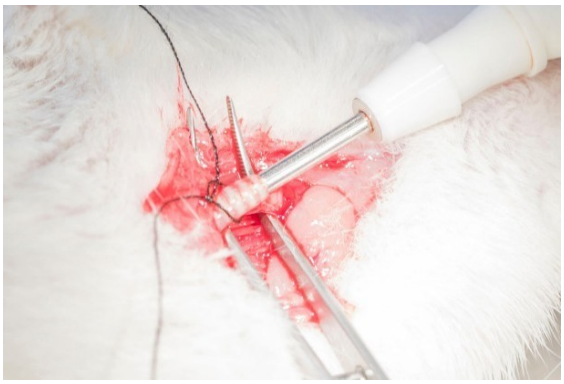

Figure 4 – Endotracheal tube inserted.

Figure 5 – Tube tied in with a double knot.

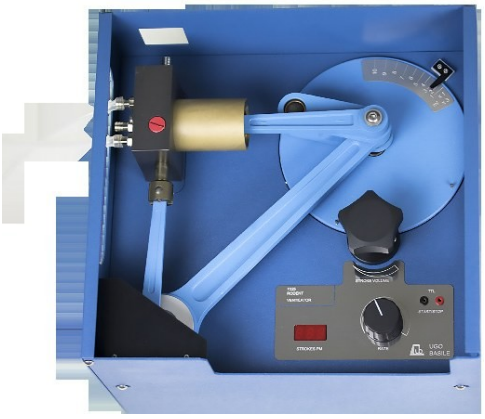

Figure 6 – Example of a rodent ventilator.

6.2 Open the Chest

1. Cut the skin along the sternum and along the lower ribs (Figure 7).
2. Open the thorax with an incision along the lower ribs (Figure 8).
3. Cut along the sternum to expose the chest cavity (Figure 9).
4. Move the chest wall over the animal's head to expose the heart.
5. Make a transabdominal incision for access to the diaphragm.

Figure 7 – Skin cut along the sternum.

Figure 8 – Incision along the lower ribs.

Figure 9 – Chest cavity exposed.

6.3 Free the Aorta

1. Dissect the aorta and remove the surrounding tissue with blunt-ended fine curved forceps (Figure 10).
2. Place a forceps underneath the aorta, grab a silk suture and pull it under the aorta (Figure 11).
3. Tie a loose knot around the aorta (Figure 12).

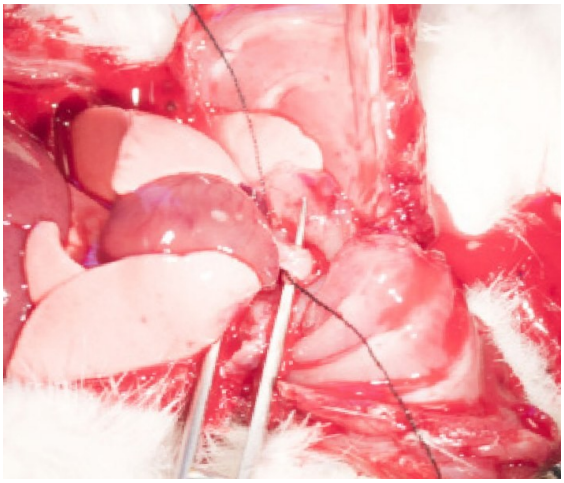

Figure 10 – Aorta freed from surrounding tissue.

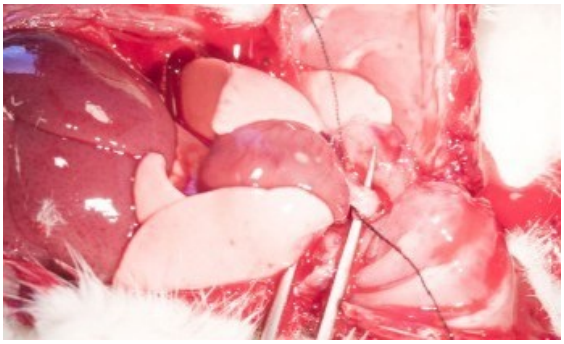

Figure 11 – Suture pulled under the aorta.

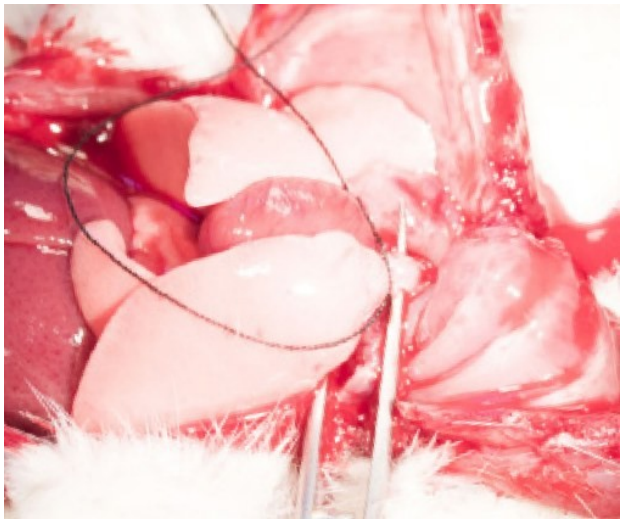

Figure 12 – Loose knot around the aorta.

6.4 Cannulate the Aorta

1. Check that the flow is on (for example 2–5 mL/min) and that perfusion fluid drips from the aortic cannula on the mounting tube kit. This minimizes the risk of air emboli.
2. Slide blunt-ended fine curved forceps under the aorta and leave them there.
3. Make a small horizontal incision in the aorta, as far up the aortic arch as possible (Figure 13). The incision must be large enough to insert the cannula.
4. Insert the aortic cannula and tie a double knot around the aorta and cannula, in the grooves of the cannula (Figure 14).
5. Coronary perfusion starts. Contractile function and a regular rhythm return. It can take a couple of minutes to reach maximum function.
6. Optional: make a small incision at the base of the pulmonary artery. This helps the coronary effluent drain from the right side of the heart.

WARNING Cut the aorta only halfway through.

Figure 13 – Small horizontal incision in the aorta.

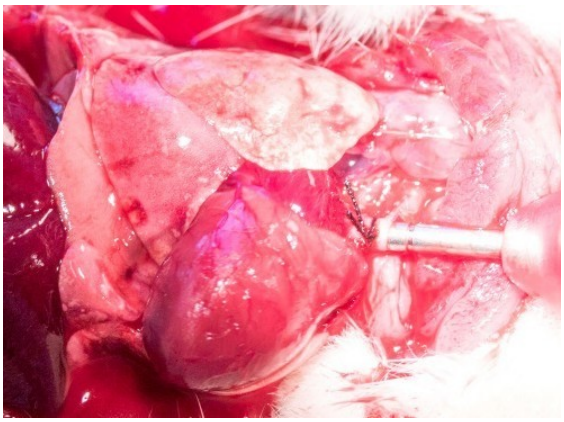

Figure 14 – Cannula inserted and tied in.

6.5 Excise the Heart

Cut the heart free together with the cannula. Keep the perfusion running while you cut, and do not pull on the aorta or the cannula.

Continue with Section 7.

7 Transfer the Heart to the 900L

The following steps are the same for both methods.

1. Rotate the heart around the cannula if needed, to place it in a suitable position.
2. Let buffer drip or run into the cannula that carries the heart before you attach it. This makes sure no air is trapped when you swap cannulas.
3. Swiftly replace the extension cannula on the aortic inlet of the 900L with the cannula that carries the heart.
4. Lower the heart into the reservoir. For the **submerged** configuration, the reservoir contains prewarmed buffer. For the **free-hanging** configuration, the reservoir is empty, and the reservoir cover must be fitted.
5. Increase the pressure or flow to the value you want for the experiment.

> **NOTE** In the submerged configuration, switch on the overflow drain before you lower the heart. See Section 5.5 of the User Manual.

8 Common Problems

Observation	Check
Heart does not recover a regular rhythm	Look for air bubbles in the cannula and aorta. Check that the cannula is not too far into the aorta and that the suture holds.
Pressure is unexpectedly high or low	Check that the cannula sits correctly, that there is no leak around the knot, and that the aortic stopcock is open.
Low coronary flow, and the heart turns a darker red	Check the position of the cannula in the aorta. If the cannula is too far into the aorta, it blocks the openings (ostia) of the coronary arteries and reduces the flow of perfusion fluid into the coronary arteries and the heart muscle. If the reduction is significant, the heart muscle can suffer from hypoxia and start to die.
Poor drainage from the right side	Consider the small pulmonary-artery incision (Section 6.4).