Sectioning of Mouse Proximal Thoracic Aorta

Jeff Z. Chen¹, Deborah A. Howatt¹, Jessica J. Moorleghen¹, Michael K. Franklin¹, Hisashi Sawada¹, Yanxiang Gao¹, Hong S. Lu¹, Alan Daugherty¹

¹University of Kentucky

ABSTRACT
This protocol is used for generation of ascending aortic serial sections for histology. This protocol generates a set of 10 slides covering ~1mm of ascending aortic tissue. Inclusion of the descending aorta provides orientation and a non-TAA control from the same animal.

EXTERNAL LINK
http://cvrc.med.uky.edu/lab-protocols

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

ATTACHMENTS
Sectioning of Mouse Proximal Thoracic Aorta.pdf

DOI
dx.doi.org/10.17504/protocols.io.be9mjh46

EXTERNAL LINK
http://cvrc.med.uky.edu/lab-protocols

PROTOCOL CITATION

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

KEYWORDS
Aorta, Histology, Cryosection, Immunohistochemistry, Immunoflorescance, Aneurysm

LICENSE
This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
MATERIALS TEXT
1. Dissecting scissors, forceps, scalpel blade, and Parafilm
2. Tissue molds 15x15 mm (Fisher Scientific Cat # 41-741) and microcentrifuge tube with screw top.
3. OCT compound (Fisher Scientific Cat # 14-373-65)
4. Probe-on-Plus microscope slides (Fisher Scientific Cat # 22-230-900)

SAFETY WARNINGS
Be careful when using the cryostat, blade is sharp.

BEFORE STARTING
1. OCT will become rubbery and difficult to cut if frozen for long periods. If you plan to cut the tissues within a few weeks, place aortic root tissues in tissue molds covered with OCT compound, wrapped tightly in parafilm, and store at -20°C. Otherwise, store aortic roots covered by OCT compound in microcentrifuge tube with screw top at -20°C for long-term storage. Label tissue molds or microcentrifuge tubes with the study name, date, and mouse ID or code as appropriate.
2. After anesthesia, cut open the thoracic cavity and draw blood from the right ventricle. Cut open the right atrium. Perfuse with saline from the apical side of the left ventricle to remove blood from the systemic circulation. A good sign for successful removal of blood from the systemic circulation is visualization of the liver turning from red to pale.

Collection of thoracic aorta

1. Euthanize mouse with ketamine : xylazine cocktail
2. Open thoracic cavity and expose heart
3. Nick right atrium with sharp scissors
4. Flush vasculature with cold saline (>5 mL) introduced slowly into the left ventricle
5. Dissect away the thymus, lungs, trachea, and esophagus
6. Inject optimal cutting temperature compound (OCT; ~0.5 mL) slowly into the left ventricle to keep patent, inject until the lumen of the descending aorta expands

Citation: Jeff Z. Chen, Deborah A. Howatt, Jessica J. Moorleghen, Michael K. Franklin, Hisashi Sawada, Yanxiang Gao, Hong S. Lu, Alan Daugherty (06/01/2020). Sectioning of Mouse Proximal Thoracic Aorta. https://dx.doi.org/10.17504/protocols.io.be9mjh46

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Dissect the heart and thoracic aorta by ligating the left/right subclavians and common carotids near the thoracic outlet and ligating the aorta above the diaphragm.

Dissect the descending aorta from the spine, taking care not to nick the aorta.

---

**Preparing tissue block**

9. Put a thin layer of OCT in mold.

10. Separate the heart from the aorta by holding the heart with the forceps and cutting with either scissors or a scalpel blade, as close to the heart as possible without causing damage.

![Figure 1: Approximate line to cut ventricle](image)

11. Cut away approximately 60 - 70% of the ventricles (the red line shown in the Fig 1).

12. Mount the heart, ascending aorta, and descending aorta with the heart facing the left and descending aorta on the right. With permanent marker, mark where the aorta lays in the mold.

13. Cover tissue with OCT. Place in cryostat with peltier on for **00:15:00** for OCT to solidify.  

---

**Preparing the tissue block and cryostat for cutting**

14. Set temperature to **-20 °C**.

15. Set chuck advance to **10 µm**.

16. Make sure stage is level.

---

**Citation:** Jeff Z. Chen, Deborah A. Howatt, Jessica J. Moorleghen, Michael K. Franklin, Hisashi Sawada, Yanxiang Gao, Hong S. Lu, Alan Daugherty (06/01/2020). Sectioning of Mouse Proximal Thoracic Aorta. [https://dx.doi.org/10.17504/protocols.io.be9mh46](https://dx.doi.org/10.17504/protocols.io.be9mh46)

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
17 Make sure sectioning starts with new region of blade or new blade

18 Make sure anti roll plate is not chipped

19 Label 10 slides / sample / region. For example: Study name, mouse ID and group, region collected (ascending or root) and number of slide (1-10)

20 Remove block from mold

21 Trim excess solid OCT from tissue, keeping intact the descending aorta

22 Cut the heart at the mid ventricle level

23 Put small drop of OCT onto mount

24 Mount trimmed tissue block with the aortic arch facing up onto holder

25 Place in peltier and wait 10 minutes for interface to solidify

Cutting Sections of the Aorta

26

The method requires cutting sequential sections in the correct orientation and without tearing, curling, or sticking incorrectly to the slide. Therefore, do not cut aortas collected from your experiments until you are sure you can reliably cut sections sequentially.

Place holder onto cryostat chuck with the heart facing down and the descending aorta on top.

27 Start sectioning, checking every 10 cycles until tissue can be seen.

Citation: Jeff Z. Chen, Deborah A. Howatt, Jessica J. Moorleghen, Michael K. Franklin, Hisashi Sawada, Yanxiang Gao, Hong S. Lu, Alan Daugherty (06/01/2020). Sectioning of Mouse Proximal Thoracic Aorta. https://dx.doi.org/10.17504/protocols.io.be9mjh46

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
28. Start collecting serial sections when arch divides into ascending aorta and descending aorta. Ascending aorta should be towards you and descending aorta should be away. This should produce sections with both the ascending aorta and descending aorta. Ascending aortas should be towards the top of the slide, descending aortas should be towards the bottom. Verify orientation of sections by counting the number of elastic laminae seen: Ascending = 8-10 lamina, Descending = 8-6 lamina.

29. Collect at least 9 serial sections per slide. (Fig 2)

![Figure 2: The organization of sections on consecutive slides](image)

Collect first section produced on bottom left corner of slide 1. Collect second section on bottom left corner of slide 2 and subsequent section in this mode. Collect eleventh section to the right of first section on slide 1. Collect serial sections by holding labeled slide downward with the label facing you. The ascending aorta should be on towards the top of the slide and the descending on bottom of slide.

30. If section rolls or flips - use antistatic brush to unroll section.

31. Stop collecting at the level of the aortic root - where cusps of valve leaflets are seen. Serial sections are collected at 10 μm/section, and 8 to 10 slides/aorta until the aortic wall disappears or is not intact anymore. Usually at least 9 serial
sections/slide can be collected.

### Clean up cryostat

32. Lock chuck wheel

33. Remove block from chuck and mount - save or discard excess tissue

34. Brush clean all surfaces with 70% ethanol and brush

35. Discard trimmings

36. Turn off light

37. Close cryostat window

### Storage

38. Slides should be stored in an appropriately labeled slide box (both the top and one of the short edges of the box) at -20°C.