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Mouse dissection and preparation of heart (aortic sinus) and brachiocephalic artery for lesion quantitation by sectioning

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We use this protocol and it's working

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Abstract

Diabetic complication:

Materials

MATERIALS

- 0.5 M EDTA ph 8.0
- 1cc Syringe with 23g needle
- Heparin (1000units /ml)
- 10cc Syringe with 30g needle
- Phosphate Buffered Saline
- Paper towels
- Dissection kit
- Anesthesia
- Base mold **Fisher Scientific Catalog #**22-038217
- Scintillation vial (20 ml)
- Tissue Tek OCT
- Formaldehyde 10% v/v in APB

Note:

Thermo Fisher Scientific **RRID:SCR_008452**

Troubleshooting

1 Prepare a 1 ml syringe by filling with 15µl 0.5 M EDTA ph 8.0 and then capping with a 23g needle. The syringe will be used for drawing blood from the heart.

2 Prepare a 10 ml syringe with 50-100 µl of heparin (1000 units per ml), then fill the syringe to 10 ml with PBS. Remove all the air from the syringe. Cap with a 30g needle. The syringe will be used to flush the heart.

3 Anesthetize the mouse.

4 Determine weight and length of the mouse.

5 Tape the mouse's arms to 3-4 layers of paper towels and place under a light source.

6 Cut the skin of the mouse from the abdomen to the top of the thorax.

7 Open the abdominal wall below the ribcage.

8 Lift the sternum with tweezers and cut the diaphragm. Then cut away the lower part of the ribcage to partially expose the heart.

9 Draw blood from the heart by sticking the needle of the 1 ml syringe (see 1) into the apex of the left ventricle. Draw blood by slowly pulling the plunger, twisting the needle. Do not draw for over a minute to avoid blood clots. Transfer the blood into an Eppendorf tube. Mix the tube by inverting it a few times. Centrifuge the tube of blood and transfer the plasma into a fresh tube.

10 Make a small incision in the right atrium for drainage.

11 Stick the 10 ml syringe into the apex of the left ventricle and flush the blood from the mouse with 10 ml of PBS.

12 If the liver is to be saved take several sections and immediately freeze at –80ºC.

13 Dry the mouse by turning it over and pressing it against paper towels. Transfer to clean, dry paper towels and re-tape the arms.

14 Place the mouse under a dissecting microscope.

15 Remove the remaining ventral part of the ribcage, carefully cut the right clavicle leaving the brachiocephalc artery and its branches intact. Using microdissection scissors, cut out all of the fat around the ascending aorta and brachiocephalic artery.

16 Cut all the fat and tissue surrounding the heart, including the pulmonary artery, and veins.

17 Flush the heart again through the right ventricle, left atrium, and left ventricle using a 10 ml syringe containing a total of 3 ml of PBS to clean out residual blood.

18 Cut the brachiocephalic artery distal to where it branches into the right subclavian and right carotid and then cut it at its branching site from the aorta so you end up with a Yshaped piece. Put it in a base mold (Fisher cat no 22-038217) and using a 10 ml syringe perfuse it with 1 ml of PBS to get it clean. It is important while handling the brachiocephalic artery to touch it only at its ends and not in the middle.

19 Cut the aorta proximal to the branching site of the brachiocephalic artery.

20 Take out the heart with the aorta. The atria must remain intact as they serve as landmarks when cutting the heart in the atherosclerosis assay.

21 Flush the heart-aorta preparation through the aorta with a 10 ml syringe containing 2 ml of PBS and place the heart in a 20 ml scintillation vial containing 10 ml of formaldehyde solution (10% v/v in aqueous phosphate buffer, Mallinckrodt).

22 Take the cleaned piece of the brachiocephalic artery and place it in a new base mold, fill it up to the first lip of the base mold with Tissue Tek O.C.T. compound. Move the brachiocephalic artery around carefully to make sure it fills with OCT.

23 Turn the Y-shaped piece of brachiocephaic artery piece so the prongs of the fork face the bottom of the base mold and freeze it in that position on a block of dry ice.