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Mouse tissue collection

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We use this protocol and it's working

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Abstract

This protocol is about collecting mouse tissues for immunohistochemistry or biochemistry experiment. While it is mainly used for brain tissues, it also can be used for other visceral tissues.

Troubleshooting

Perfusion

1 Mice are anesthetized with Avertin (1mL/25g body weight).

2 Immobilize a mouse and cut through the skin and muscles in the lower abdomen exposing the liver using a sharp scissors.

3 Cut through the diaphragm, then down the right and left sides of the chest, through the ribs, until the heart is fully visible.

4 Grasp the heart firmly near the apex with a blunt forceps, then insert the butterfly needle gently into the left ventricle.

5 Using a small, sharp scissors, make a small cut in the right atrium to serve as a drain.

6 Flow 1x PBS (Quality Biological) containing 1 IU Heparin/mL (about 00:00:30) followed by [M] 4 % volume paraformaldehyde for fixation until evidence of stiffness of tissues is observed. 30s

7 Tissues are collected and post-fixed in [M] 4 % volume PFA at 4°C for about 24:00:00 1d

8 For floating sections, tissues are transferred to 30% sucrose for cryoprotection, then kept at 4 °C for about 48:00:00 until tissues sink to the bottom of the vessel. 2d

9 The floating sections are collected with microtome combined with freezing unit. After getting 30~40 um thickness of floating sections, they can be stored in PBS-sodium azide ([M] 0.05 % volume) solution for 1344:00:00 maximum. 8w

10 For paraffin sections for histopathology evaluation, tissues are transferred to [M] 70 % volume ethanol after fixation then kept at 4 °C until usage.

Frozen tissues

11 Tissues for biochemical experiment such as gene expression analysis, protein assay or lipidomics are collected using snap-freezing in dry ice without perfusion, then stored at -80 °C ^{o} C.