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RNA Extraction From Mouse Tissue

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Protocol status: Working
We use this protocol and it's working

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Abstract

how to extract RNA from mouse tissue

Materials

RNASE-ZAP
pentobarbital sodium and phenytoin sodium
DNA LO BIND 1.5 mL tubes
Qiagen Rneasy Mini Columns Protocol (Cat No 74104, Qiagen)
DPBS

Preparation before Perfusion

1 Before beginning perfusion make sure all surfaces and tools are sprayed down with 70% ethanol followed by RNASE-ZAP.

2 Ensure perfusion pump is PFA free, DPBS is sterile filtered and on ice, and the surface used to perfuse the mouse is also sterilized with 70% ethanol followed by RNASE-ZAP

3 Get a bucket of dry ice to snap freeze the extracted tissue

4 Get two cooling blocks - should be at least 0 degrees. Put Petri dishes full of sterile DBPS on top of the cooling blocks.

5 Prepare a 50 ml tube with RNASE-ZAP. Put your tools in this tube while you wait to begin the perfusion.

6 Label DNA LO BIND 1.5 mL tubes with the appropriate label for the mouse and tissue you want to convert into RNA.

Mouse Perfusion with only DPBS

7 Begin the perfusion as normal - Anesthetize mouse using pentobarbital sodium and phenytoin sodium. A 30g mouse would need roughly 100 ul of this pentobarbital sodium and phenytoin sodium solution.

8 Ensure deep anesthesia with paw pinch. Mouse should not respond.

9 Using four 26g needles, secure mouse paws to styrofoam.

10 Make an incision along the abdomen, extending up to the rib cage.

11 Expose chest cavity and secure with hemostats clamped to the xiphoid process.

12 Insert 22g needle into the left ventricle and hold via hemostat.

13 Make small incision in the right ventricle.

14 Immediately start peristaltic pump connected to sterile filtered DPBS, flowing at a rate of 10 mL/min

15 Once the perfusate runs clear, stop pump

Extracting tissue

16 Remove the tissue needed from the animal and place into the DBPS on the cooling core. For example: if we want to extract RNA from the duodenum, the animals intestinal track would be removed from the carcass and placed into the DPBS on the cooling core

17 Make small precise cuts to remove roughly 30mg of tissue and place into DNA LO BIND 1.5 mL tubes.

18 Put DNA LO BIND 1.5 mL tubes straight into the dry ice to snap freeze the tissue.

19 Repeat for however many tissues you want to convert into RNA.

20 The tissue can be stored at -80°C until ready to convert the tissue into RNA.

Qiagen Rneasy Mini Columns Protocol

21 From here on we follow the Qiagen Rneasy Mini Columns Protocol (Cat No 74104, Qiagen) for converting animal tissue into RNA.