Sep 26, 2024 Version 3

Mouse brain, gut and plasma collection V.3

DOI
dx.doi.org/10.17504/protocols.io.14egn3pkzl5d/v3

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DOI: dx.doi.org/10.17504/protocols.io.14egn3pkzl5d/v3

Protocol Citation: Livia Hecke Morais 2024. Mouse brain, gut and plasma collection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn3pkzl5d/v3Version created by **Catherine Oikonomou**

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

Created: March 13, 2024

Last Modified: September 26, 2024

Protocol Integer ID: 108401

Keywords: ASAPCRN

Funders Acknowledgement:
ASAP
Grant ID: ASAP-020495

Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

Protocol used in the Mazmanian lab for collecting brain and gut tissues and plasma from mouse for metabolomics.

Note that any protocol involving animals should be approved by your Institutional Animal Care and Use Committee (IACUC) before use.

Protocol materials

Precellys 2mL Soft Tissue Homogenizing Ceramic Beads **Cayman Chemical Company** **Catalog #**10011152

In 2 steps

Safe-Lock Tubes 2 ml Biopur (preferably packed individually) **Eppendorf** **Catalog #**0030121597 Step 24

Vacutainer Hemogard Closure Plastic K3-EDTA Tube **BD Biosciences** **Catalog #**367836 Step 28

Thermo Scientific Pierce Microcentrifuge Tubes, 1.5 mL **Thermo Fisher Scientific** **Catalog #**69715 Step 30

Mouse brain, gut and plasma collection

10m

1 Euthanize the mouse inside a fume hood by decapitation using scissors (or by alternative method approved by your IACUC).

2 Use straight scissors to separate the head from the rest of the body.

3 **For brain samples**

4 Use a surgical blade or straight scissors to rapidly remove the brain from the skull by severing the proximal end of the spinal cord from the occipital bone of the skull

5 Use forceps, a Boehler bone cutter, and serrated forceps to detach any residual muscle tissue from the posterior and inferior portions of the skull.

6 Place the brain in an ice-chilled stainless steel coronal matrix.

7 Use a single-edge razor blade to section the brain tissue in slices of approximately 1 mm .

8 Dissect brain regions of interest according to a reference brain atlas.

> **Note**
>
> For striatum and motor cortex, we use slices from +1.54 mm to +0.10 mm anterior-to-posterior relative to bregma. For substantia nigra, we use slices from -2.70 mm to -3.52 mm relative to bregma. For brainstem, we start at the superior colliculus of the midbrain and cut obliquely to meet the pituitary gland.

9 Weigh samples and record for future reference.

10 Add samples to

Precellys 2mL Soft Tissue Homogenizing Ceramic Beads **Cayman Chemical Company Catalog #**10011152

11 Snap-freeze on dry ice.

12 Store samples at -80 °C until use.

13 **For gut samples**

14 Using large scissors and large forceps, make an incision through the abdominal skin.

15 Sterilize scissors by spraying with [M] 70 % (v/v) EtOH.

16 Insert the sterilized scissors below the skin to expose the abdominal muscle.

17 Make another incision through the abdominal muscle to access the internal organs.

18 Remove the intestine from the duodenum to the rectum.

19 On an ice-chilled dissection tray, dissect desired intestinal samples.

> **Note**
>
> For the small intestine, we dissect from 1 cm distal to the stomach to 1 cm proximal to the cecum. For duodenum, we dissect from 2 cm distal to the pyloric sphincter. For ileum, we take from 2 cm proximal to the cecum. For large intestine, we dissect from distal of the cecum to 10 mm proximal to the anus. For distal colon, we dissect 1 cm proximal to the rectum.

20 To sample contents of small intestine, cecum, and distal colon, remove contents using a 5 mL syringe and 14 G needle.

21 Cut tissue samples into 1 cm sections.

22 Weigh samples and record for future reference.

23 Add tissue samples to

Precellys 2mL Soft Tissue Homogenizing Ceramic Beads **Cayman Chemical Company Catalog #**10011152

24 Add tissue content samples to sterile

Safe-Lock Tubes 2 ml Biopur (preferably packed individually) **Eppendorf Catalog #**0030121597

25 Snap-freeze samples on dry ice.

26 Store samples at -80 °C until use.

27 **For plasma samples**

28 At Room temperature collect trunk blood in

Vacutainer Hemogard Closure Plastic K3-EDTA Tube **BD Biosciences Catalog #**367836

29 Separate plasma by centrifugation at 2.500 x g for 00:10:00 10m

30 Transfer plasma to a pre-cooled

Thermo Scientific Pierce Microcentrifuge Tubes, 1.5 mL **Thermo Fisher Scientific Catalog #**69715

31 Store at -80 °C until use.