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Mouse brain, gut and plasma collection V.3

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Protocol status: Working

We use this protocol and it's working

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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**

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

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

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

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  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.