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# Mouse brain, gut and plasma collection V.2

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

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## Disclaimer

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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. Protocol has been approved by the California Institute of Technology's Institutional Animal Care and Use Committee (IACUC).

## Mouse brain, gut and plasma collection

1 At 4 months of age, mice is euthanized inside chemical hood by decapitation using scissors (as approved by IACUC protocol)

2 The head is separated from the rest of the body for brain and gut dissections using straight scissors

3 **For brain dissection**

4 The brain is rapidly removed from the skull by serving the proximal end of the neck and vertebrae from the occipital bone of the skull using a surgical blade or straight scissors.

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

6 Brain is placed in an ice-chilled stainless steel coronal matrix.

7 Brain tissue is sectioned in slices of approximately 1 mm using single edge razor blades.

8 Substantia nigra, striatum, motor cortex(referred to as cortex) and caudal brainstem (referred to as brainstem) were dissected within three minutes using a brain atlas as reference[1] . For striatal brain slices spanned from anterior to posterior (AP) +1.54 mm to +0.10 mm relative to bregma. Motor cortex was taken from same slices as to striatum. Substantia nigra slices were taken from anterior to posterior (AP) -2.70 mm to -3.52 mm relative to bregma. Brainstem was dissected starting at superior colliculus of idbrain and cut obliquely to meet the pituitary gland.

9 All tissue samples were weighed, stored in and stored at Precellys® 2 mL Soft Tissue Homogenizing Ceramic Beads tubes (Catalog number: 10011152), snap-frozen in dry ice and stored at -80°C
until processing.

10 References:
1. Paxinos, G. and Franklin, K.B.J. (2001) The Mouse Brain in Stereotaxic Coordinates. 2nd Edition, Academic Press, San Diego.

11 **Gut samples collection**

12 Using large scissors and large forceps, an incision is made into abdominal skin

13 Next, sprayed scissors with 70% EtOH is inserted below skin to expose the abdominal muscle.

14 Another incision is made into the abdomen muscle to access internal organs.

15 The intestine from duodenum to rectum is removed.

16 All intestinal tissue samples were sized to 1 cm

17 The small intestine is separated by cutting 1 cm distal to stomach and until 1 cm proximal to cecum.
Duodenum samples were taken from 2cm distal to pyloric sphincter. Ileum is taken 2 cm proximal to cecum.

18 Small intestine content is removed using a 5 mL syringe and 14 G needle

19 Cecum is separated and cecum content is removed using a 5 mL syringe and 14 G needle

20 Large intestine was taken by cutting distal to cecum and 10 mm proximal to anus.

21 Only distal colon was taken by cutting the portion 1 cm proximal to the rectum.

22 Distal colon content was removed using a 5 mL syringe and 14 G needle

23 All tissue samples were weighed, and stored at Precellys® 2 mL Soft Tissue Homogenizing Ceramic Beads tubes (Catalog number: 10011152), snap-frozen in dry ice and stored at -80°C until processing. Tissue contents were stored in sterile 2.0mL Safe-Lock Microcentrifuge tube.

24 **Trunk blood collection and plasma**

25 After Trunk blood was collected in BD Vacutainer™ Hemogard™ Closure Plastic K3-Edta Tubes (Catalog number: 15349700) and kept at room temperature before plasma separation.

26 Plasma was separated by centrifugation at 2,500 x g for 10 minutes

27 Plasma was transferred to a pre-cooled collection vial Thermo Scientific Pierce 1.5 mL Capacity Microcentrifuge Tubes (Catalog number: 69715) and stored at -80°C until processing