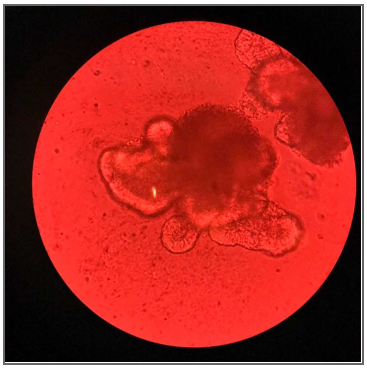

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Mouse genetic models to manipulate enterochromaffin cell activity - Murine Organoid ELISA

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ABSTRACT

This protocol describes how we maintain murine intestinal organoids and perform the enzyme-linked immunosorbent assay (ELISA) for serotonin (5-HT).

MATERIALS

DPBS, no calcium, no magnesium (Thermo Fisher #14190144)
DPBS, no calcium, no magnesium + 2 mM EDTA
Advanced DMEM/F-12 (Thermo Fisher #12634010)
1 M HEPES (Thermo Fisher #15630080)
GlutaMAX™ Supplement (Thermo Fisher #35050061)
Penicillin-Streptomycin (10,000 U/mL) (Thermo Fisher #15140163)
Recombinant Murine Noggin (Peprotech #250-38)
Mouse EGF Recombinant Protein (Thermo Fisher #PMG8041)
N-Acetyl-L-cysteine (Millipore-Sigma #A7250-5G)
B-27™ Supplement (50X), serum-free (Thermo Fisher #17504044)
R-spondin 2 (supernatant from R-spondin expressing HEK293 cells)
Corning® Matrigel® Matrix (Corning #356231)
Bovine Serum Albumin (Millipore-Sigma #A9418)
Serotonin ELISA kit (LDN #BA-E-8900)
24-well plate (Falcon® 24-Well Plate #38021)
Corning™ Falcon™ Cell Strainers 70 µm (Corning #08-771-2)
Ringer's solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES-Na [pH 7.4]))

Following four mouse lines were used.
RC::FL-hM3Dq (Jackson Labs, Strain #026942)
Tac1Cre (Jackson Labs, strain #021877)
Pet1Flp (gift of Dr. Susan Dymecki)
RC::PFTox (gift of Dr. Susan Dymecki)

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

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Keywords: Intestinal organoid, Serotonin, 5-HT, ELISA

Preparation or basal media

10m

1 Add 5 mL of GlutaMAX, 1 M HEPES, and Penicillin-Streptomycin to 500 mL of Advanced DMEM/F-12 and filter through 0.22 um. This basal media can last up to1 month.

Preparation of complete organoid culture media

2 Add 800 µL of B-27, Recombinant Murine Noggin (final conc. 100 ng/mL), Mouse EGF Recombinant Protein (final conc. 500 ng/mL), *N*-Acetyl-L-cysteine (final conc. 0.5 mM), and 4 mL of R-spondin 2 to 40 mL of basal media. This complete media can last up to 2 weeks.

Mouse organoid prep

10m

3 Organoids were generated from male Tac1-Cre(+/-);Pet1Flp(+/-);RC::FL-hM3Dq(+/-) or Tac1-Cre(+/-);Pet1Flp(+/-);RC::OFTox(+/-) animals (5-8 weeks old).

4 Euthanize the animal under CO_2 and spray belly with 70% EtOH.

5 Isolate the whole small intestine and divide the tissue into three pieces. Use the middle piece (jejunum) for organoid generation.

6 Filet open the intestine along the mesentery.

7 Scrape off the villi using a glass coverslip. Scrape well so that all the villi come off.

8 Cut the intestine into 2-4 mm pieces and move to a 50 mL falcon tube.

9 Add 10 mL ice-cold DPBS. Pipette up and down with 10 mL pipettes.

10 Wait for a couple of minutes until the tissue settles down and discard the supernatant.

11 Repeat the step 9-10 until the supernatant becomes clear (usually 4-5 times).

12 Add 30 mL of ice-cold DPBS + 2 mM EDTA and rock the tube at 4°C for 30 minutes.

13 While waiting, prepare 6x 50 mL falcon tubes and label them No.1-6.

14 Wait for a couple of minutes until the tissue settles down and discard the supernatant.

15 Add 10 mL ice-cold DPBS. Pipette up and down with 10 mL pipettes.

16 Wait for a couple of minutes until the tissue settles down and move the supernatant to falcon tube #1 prepared at step #13.

17 Repeat the step 15-16 five times. Note that the supernatant should be put into separated tubes prepared at step #13 (tube #2-6).

18 Place 20 µL of each supernatant fraction on a glass slide and check under a microscope.

19 Identify fractions that contain crypts.

20 Combine crypts-containing fractions and filter through a 70 µm strainer.

21 Spin down at 900 g for 5 minutes and discard the supernatant.

22 Resuspend pellet with 10 mL ice-cold basal media and spin down at 900 g for 5 minutes.

23 Carefully remove all the supernatant.

24 Repeat the step #22 and #23.

25 Resuspend the pellet into 400 µL ice-cold matrigel.

26 Pipette 50 µL of the matrigel/organoid suspension into the center of each of four wells of a prewarmed 24-well plate to form domes in the center of each well.

27 Place the lid on the culture plate and quickly turn the plate upside down.

28 Incubate at 37°C for 10 minutes to set the matrigel.

29 Turn the plate back upside so that you can apply media to wells. Gently add 500 µL complete media to each well.

30 Add basal media to surrounding wells to prevent the wells from drying.

Maintenance of intestinal organoids

46m

31 Put an aliquot of matrigel in fridge at 4°C. Put a 24-well plate to a 37°C incubator. 1m

32 Put desired volume of complete media at 37°C 1m

33 Aspirate media from wells 2m

34 Add 500 µL cold basal media to a well and let it sit for about 15 seconds 2m

35 Pipette up and down to dissolve matrigel, collect organoids, and move to a 15 mL falcon tube 3m

36 Add 500 µL cold basal media to a well, collect residual organoids, and move to the same 15 mL falcon tube 3m

37 Place a 200 µL tip inside a 1000 µL tip and pipette up and down ~25 times to break up organoids 3m

38 Add 10 mL cold basal media to the falcon tube and mix well 1m

39 Centrifuge at 200 g for 3 min at 4°C 3m

40 Aspirate the supernatant (be careful not to aspirate the organoid pellet) 2m

41 Add 10 mL cold basal media to the falcon tube and mix well 1m

42 Centrifuge at 200 g for 3 min at 4°C 3m

43 Aspirate the supernatant (be careful not to aspirate the organoid pellet) 1m

44 Add 200 µL of ice-cold matrigel to the falcon tube and mix the organoids well 2m

45 Pipette 50 µL of the matrigel/organoid suspension into the center of each of four wells of a prewarmed 24-well plate to form domes in the center of each well 3m

46 Place the lid on the culture plate and quickly turn the plate upside down 1m

47 Incubate at 37°C for 10 minutes to set the matrigel 10m

48 Turn the plate back upside so that you can apply media to wells. Gently add 500 µL complete media to each well 2m

49 Add basal media to surrounding wells to prevent the wells from drying 2m

50 Exchange the media every 3-4 days or whenever the media turns yellow.

51 Split again in 6-8 days.

Preparation of organoids for ELISA 38m

52 Organoids are grown for 4-6 days

53 Aspirate the supernatant (be careful not to aspirate the organoid pellet) 2m

54 Add 500 µL of cold DPBS to a well and let it sit for about 15 seconds 1m

55 Gently pipette up and down ~10 times with P-1000 to remove matrigel (but not to break the organoids up) and move to a 15 mL falcon tube. 2m

56 Add 500 µL of cold DPBS to the tube 2m

57 Put the tube on ice for 5 min 5m

58 Gently pipette up and down ~10 times with P-1000 2m

59 Add 10 mL cold DPBS and spin down at 200 g for 3 min at 4°C 3m

60 Aspirate the supernatant as much as possible 1m

61 Add 1 mL cold DPBS and gently pipette up and down ~10 times with P-1000 2m

62 Add 10 mL cold DPBS and spin down at 200 g for 3 min at 4°C 3m

63 Aspirate the supernatant as much as possible 1m

64 Repeat the step #33-35 twice to completely wash the matrigel out 12m

65 Add 100 µL DPBS + 0.1% BSA and move organoids to an eppendolf tube (it is important to add 0.1% BSA to prevent organoids from sticking to tubes) 1m

66 Remove the supernatant and add 100 µL Ringer's (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES-Na [pH 7.4])) buffer for ELISA assay (do not lyse the organoids as you are measuring released serotonin). 1m

5-HT ELISA

67 ELISA is performed according to the manufacturer's protocol (https://www.ldn.de/wp-content/uploads/IFU-BA-E-5900R-V15.0_wz.pdf).