

Mouse intestinal tissue is dissected, flushed with PBS to remove luminal contents, and placed in PBS on ice. Mesentery and pancreatic tissue are removed, then intestinal tissue is cut into 1.0-1.5cm segments. Tissue segments are individually slid onto a borosilicate glass Pasteur pipette and, using pressure from a finger for stability and under microscopy, forceps are used to create a thin nick along the serosa and muscle layers. A cotton swap is wetted with cold PBS and used to wipe along the nick while slowly turning the tissue using the stabilizing finger to peel away the longitudinal muscle and myenteric plexus layer. Collected tissue is immediately placed in a digestive KREBS solution buffer with 1.3 mg/mL collagenase type II (Worthington, LS004176) and 0.3 mg/mL BSA (Sigma, A4161). KREBS solution is composed of the following: 121 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 10 mM HEPES, 21.2 mM NaHCO₃, 8 mM glucose. All tissues are collected and placed into the solution on ice within 30 minutes of dissection.

The digestive KREBS solution is moved to 37 °C and bubbled with carbogen for 1 hour. Following this hour, solution is quenched with base media and centrifuged at 356 g for 10 minutes at 4 °C. Enteric glial cell media composition is outlined below. The remaining pellet is gently resuspended in enteric glial cell media supplemented with 1x CloneR (Stem Cell Technologies, 5889) and 50ug/mL primocin (Invivogen, ant-pm-1), filtered through 100 uM filters (Fisher Scientific, 08-771-19), and plated onto 10cm 0.1mg/mL poly-ornithine primed, 10µg/mL laminin coated plates. Media is replaced every other day and cells are split using Accutase at room temperature after reaching confluence or 2 weeks in culture. Enteric glia from *Plp1*-eGFP mice retain eGFP expression, enteric glia morphological diversity, and respond to cytokine stimulation by upregulating reactive glia markers.

Media Formulations and Culture Conditions

Plating

1:1 DMEM/F12 + Neurobasal
1x Pen/Strep
1x N2-Max
1x B27, serum free
20ng/mL FGF2
10ng/mL GDNF
50ng/mL NGF
50ug/mL primocin
1x CloneR

Maintenance

1:1 DMEM/F12 + Neurobasal
1x Pen/Strep
1x N2-Max
1x B27, serum free
20ng/mL FGF2
10ng/mL GDNF
50ng/mL NGF

0.1mg/mL poly-ornithine primed, 10ug/mL laminin 10cm plates
(2 mice per plate)