Reagents
CRITICAL: The list provides details of the reagents used in our laboratory, however many reagents can be substituted with similar commercially available alternatives.

ANIMALS
- Typically we use 8-week old male Sprague-Dawley rats (Charles River Ltd, Kent, UK) to obtain retinal tissue for explant culture; however, tissue from Wistar, Lewis, and Brown Norway rats have produced comparable results in our hands. CAUTION: ensure all relevant local, and national, ethical and legal guidelines/regulations associated with the use of experimental vertebrate animals are followed.

GENERAL REAGENTS
- Nunc 24-well multi-dish plates, flat bottom (VWR International, Lutterworth, UK; catalogue number 142475)
- Antibiotic-antimycotic (100X) solution (Invitrogen Inc., Paisley, UK; catalogue number 1540062)
- B-27 Supplement (50X), liquid (Invitrogen Inc.; catalogue number 0080085-SA)
- 7 ml sterile unlabelled Bijou tube (Sterilin Ltd UK, Newport, UK; catalogue number 129A)
- 24x60 mm glass coverslips (VWR International; catalogue number 737-0074)
- Sterile distilled water
- Dulbecco’s Phosphate Buffered Saline (PBS) with MgCl\(_2\) and CaCl\(_2\), liquid, sterile-filtered (Sigma-Aldrich UK, Gillingham, UK; catalogue number D8662-500mL)
- Ethanol absolut (Sigma-Aldrich UK; catalogue number 32221)
- 150 mm diameter filter paper, Grade 1 (Whatman International Ltd., Banbury, UK; catalogue number 1001-150)
- 320 mm diameter filter paper, medium retention, medium/fast flow rate cellulose (Fisher Scientific, Loughborough, UK; catalogue number FB59035)
- FluorSave Reagent (20 mL) (Merck Chemicals Ltd., Beeston, UK; catalogue number 345789)
- 200 mM L-glutamine solution (100X), liquid (Invitrogen Inc.; catalogue number 25030032)
- Goat serum (Invitrogen Inc.; catalogue number 16210072)
- Hanks’ Balanced Salt Solution (1X), liquid containing Ca\(^{2+}\) and Mg\(^{2+}\) (HBSS; Invitrogen Inc.; catalogue number 24020091)
- ImmEdge Pen (Vector Laboratories, Peterborough, UK; catalogue number H-4000)
- Millicell Cell Culture Inserts (12 mm diameter; 0.4 \(\mu\)m pore) (Millipore UK Ltd., Dundee, UK; catalogue number PICM01250)
- N-2 Supplement (100X), liquid (Invitrogen Inc.; catalogue number 17502048)
- Neurobasal®-A Medium (1X), liquid (Invitrogen Inc.; catalogue number 10888022)
- Optimal cutting temperature (OCT) embedding compound (Tissue-Tek brand, product 4583; VWR International; catalogue number 25608-930)
- Paraformaldehyde, powder (Sigma-Aldrich UK; catalogue number 17502048)
- Peel-A-Way Mould, 22x22 mm truncating to 8 mm\(^2\) (TAAB Laboratories Equipment Ltd., Aldermaston, UK; catalogue number E113)
• Penicillin (10,000 U/mL)-Streptomycin (10,000 µg/mL) Solution, liquid (Invitrogen Inc.; catalogue number 15140122)
• 90 mm plastic Petri dish (Sterilin Ltd UK; catalogue number 101R20)
• Sodium chloride (Fisher Scientific; catalogue number S/3160/53)
• Sodium dihydrogen orthophosphate, dihydrate (Fisher Scientific; catalogue number S/3760/53)
• di-Sodium hydrogen orthophosphate, dihydrate (Fisher Scientific; catalogue number S/4440/53)
• Sodium hydroxide, anhydrous pellets (Sigma-Aldrich UK; catalogue number S5881)
• Sucrose (Sigma-Aldrich UK; catalogue number S9378)
• Superfrost Plus microscope slides (VWR International; catalogue number 631-0108)
• 20 ml syringe (BD, Oxford, UK; catalogue number 300613)
• 0.45 µm pore Minisart® sterile syringe filter (Sartorius Stedim Biotech, Epsom, UK; catalogue number 16555)
• Triton X-100 (Sigma-Aldrich UK; catalogue number X100)
• Trypsin-EDTA (10X), liquid (0.5% trypsin, 5.3 mM EDTA) (Invitrogen Inc.; catalogue number 15400054)

Reagent Setup

70% ethanol: To make 1 L, add 700 ml ethanol absolut to 300 ml distilled water and mix.

Dissection medium: Add 5 ml of the antibiotic-antimycotic (100X) solution to 500 ml HBSS and mix. CRITICAL: to ensure sterility of medium this step must be performed in the laminar flow hood using good sterile technique. Store protected from light at 4°C.

Explant culture medium: To make up 20 ml, mix together 19 ml Neurobasal®-A Medium, 400 µl B27 supplement, 200 µl N2 supplement, 200 µl penicillin/streptomycin solution and 80 µl L-glutamine solution. Filter sterilise medium using 20 ml syringe equipped with 0.45 µm pore syringe filter. CRITICAL: to ensure sterility of medium this step must be performed in the laminar flow hood using good sterile technique. Store medium protected from light for a maximum of 2 days at 4°C.

10M sodium hydroxide solution: To make 100 ml, dissolve 4 g sodium hydroxide pellets in about 80 ml distilled water; once dissolved make up volume to 100 ml using distilled water. Store at room temperature. CAUTION: dissolution of sodium hydroxide in water is an exothermic process, this step should always be performed in a fume cupboard and care should be taken to add the sodium hydroxide pellets to the water slowly to ensure the solution does not boil. CAUTION: sodium hydroxide pellets and solution are highly caustic, therefore use appropriate PPE (including gloves and safety goggles) and take care to ensure no skin contact; for guidance see MSDS.

10X phosphate buffered saline (PBS) solution: To make 5 L, dissolve 425 g sodium chloride, 53.15 g di-sodium hydrogen orthophosphate and 19.5 g sodium dihydrogen orthophosphate in 4 L distilled water. Once completely dissolved, use
distilled water to make volume up to 5 L and adjust pH to 7.4. Store at room temperature.

4% paraformaldehyde/0.1M PBS solution (4% PFA): To make 1 L, heat 800 ml distilled water to approximately 60°C, add 1 ml 10M sodium hydroxide solution and dissolve 40 g paraformaldehyde powder with continuous mixing over heat. Once fully dissolved, allow solution to cool to room temperature and add 100 ml 10X PBS solution, mix and then add distilled water to adjust volume to 1 L total. Finally, adjust pH to 7.2-7.4, filter through 320 mm diameter medium/fast flow rate filter paper (or equivalent) and store at 4°C for up to 1 week. CAUTION: paraformaldehyde powder and solution is toxic, weighing of PFA powder and preparation of solution must always be performed in a fume cupboard. Use appropriate PPE and take care to ensure no skin contact; for guidance see MSDS.

0.1M PBS (1X) solution: To make 1 L, add 100 ml 10X PBS solution to 900 ml distilled water and mix. Check pH is 7.4. Store at room temperature.

30% sucrose solution: To make 100 ml, dissolve 30 g sucrose in about 70 ml 0.1M PBS solution; once dissolved, make up volume to 100 ml total by adding 0.1M PBS solution. Store at 4°C, do not use once contaminated with microorganisms (this can be prevented by filter sterilisation).

PBS-T (0.1M PBS with 0.2% triton X-100) solution: To make 100 ml, add 200 µl triton X-100 solution to 100 ml 0.1M PBS solution while stirring. Store at room temperature.

Antibody blocking solution: To make 100 ml, add 5 ml goat serum to 95 ml PBS-T solution and mix. Store at 4°C, do not use once contaminated with microorganisms (this can be prevented by filter sterilisation).