Slice and Explant Culture Protocols
Mitchell Lab 2008

embyro age E11.5 – E16.5 (adapted from Hevner, Rubenstein and Price lab protocols)

1. Setup
   Prepare the following:-
   1X Krebs (1000 ml) and cool on ice.
   100 ml of *sterile filtered* Krebs buffer and cool on ice.
   100 ml *serum–supplemented* medium and incubate at 37°C and 5% CO_2.
   100 ml *serum–free* medium and incubate at 37°C and 5% CO_2.
   50 ml of 4% low melt agarose in PBS with glucose (5 mg/ml).

2. Sacrifice pregnant female by cervical dislocation, remove the embryos and place them in ice–cold Krebs buffer in a Petri dish on ice.

3. Working in ice–cold Krebs buffer, dissect the brains and embed them in agarose (cool on ice to set).

4. After the agarose hardens, trim block for sectioning. For intact thalamic tracts sectioning should be done 55º from sagittal plain.

5. Fill Vibratome well with ice-cold Krebs buffer, and surround well with ice. (Change the Krebs buffer and add more ice between brains.)

6. Cut thick sections (300 μm) and transfer to sterile ice-cold Krebs buffer in a sterile Petri dish on ice using a sterile paintbrush.

7. Select the sections to be used for experiments and transfer them onto polycarbonate membranes (Nunc #137060), on the surface of serum–supplemented medium (1 ml) in 6-well culture plates. Slices must be in incubator within two hours of initial dissection.

8. After incubating slices in serum–supplemented medium for 1-2 hrs, exchange for serum–free medium and place back in the incubator.

9. Slices can now be used for cell or explant culture, for DiI labeling or for heterotopic slice culture.

10. Sections are transferred to sterile ice-cold Krebs buffer for microdissection using microsurgical knife (WPI Inc. #500249). Regions of interest are isolated for dissociation, for preparation of explants (manually cut into explants of 150-200μm), or sections are transected (e.g. diencephalon-telencephalon boundary) and reconstructed from constituent regions of different genotype (transfer diencephalon/telencephalon onto fresh polycarbonate membrane using pipette and position with fine forceps before aspirating buffer to leave tissue on dry membrane).

11. Slices or explants can be maintained in vitro for 3-4 days, after which contamination and cell death become limiting.

Solutions and media

10X Krebs buffer (autoclave; can be stored at 4°C for 2-3 months)

NaCl	73.6 g/l	(126 mM at 1X)
KCl	1.87 g/l	(2.5 mM at 1X)
NaH2PO4•H2O	1.66 g/l	(1.2 mM at 1X)
MgCl2	2.44 g/l	(1.2 mM at 1X)
CaCl2	3.68 g/l	(2.5 mM at 1X)

1X Krebs buffer (use same day as prepared)

10X Krebs	100 ml/l	
glucose	1.98 g/l	(11 mM)
NaHCO3	2.1 g/l	(25 mM)
dilute to 1000 ml		

Sterile filtered Krebs buffer (use same day as prepared)

1X Krebs buffer	98 ml
HEPES 1M	1 ml
Pen/strep 100X	1 ml
Sterile filter at pore size <1 μm	

Serum–supplemented medium (use same day as prepared)

MEM with glutamine	88 ml	(MEM = modified Eagle's medium; Gibco)
fetal calf serum	10 ml	
50% glucose	1 ml	
Pen/strep 100X	1 ml	

Serum–free medium (use same day as prepared)

Neurobasal medium	95 ml	(Gibco)
B-27 supplement	2 ml	(Gibco)
50% glucose	1 ml	
100X glutamine	1 ml	
Pen/strep 100X	1 ml	

50% glucose (filter sterilize into 10 ml Falcon tube; store at 4°C for up to 1 wk)

5 g glucose
H2O with stirring to 10 ml final volume.