

SLICE CULTURE PROTOCOL

THE DAY BEFORE, MAKE SURE YOU HAVE:

- Sterile H₂O at 4°C.
- 10x Krebs and all other reagents.
- 100 ml 4% LMP agarose in sterile PBS. Boil until melted and leave it at 43°C overnight.

BEFORE YOU START:

- Add **0.99 g** Glucose and **1.05 g** NaHCO₃ (sodium bicarbonate) to 450 ml of sterile H₂O at 4°C.
- Keep Krebs on ice as long as possible; otherwise some salts go out of solution.
- Take stock reagents to the tissue culture hood:
 - From 4°C: 10x Krebs, Hepes, Gentamicin, 50% glucose, MEM, Neurobasal.
 - From -20°C (defrost in water bath): Pen-Strep, FCS, B27, Glutamine.
- From now on, prepare all reagents on tissue culture hood:
 - Add **50 ml** 10x Krebs to the 450 ml to prepare 1x Krebs.
 - Filter in 0.2 µm pore.
 - Prepare **50 ml** of Sterile filtered 1x Krebs.
 - Prepare **50 ml** of MEM medium following the protocol.
 - Prepare **50 ml** of Neurobasal medium following the protocol.

SLICE PREPARATION

- **Remove** embryos and place them in ice cold Krebs in a petri dish.
- **Remove** brains and embed them in LMP agarose. Place the molds in a cold water bath at 4°C for hardening.
- **Cut** 250 µm sections in a vibratome. Cortex oriented towards the razor. The vibratome well contains ice cold Krebs which is frequently exchanged for fresh cold Krebs.
- **Collect** sections into petri dishes containing sterile filtered Krebs at 4°C.
- **Transfer** the section in the hood to polycarbonate culture membranes in Falcon organ tissue culture dishes containing 1 ml MEM medium. Place them into the incubator for 1 h (37°C, 5% CO₂).
- **Change** to 1 ml Neurobasal medium.

- If needed, label with **BrdU** 1 μ l/ml from 10 mg/ml stock (for BrdU-labeled transplants see Anderson et al., 2001).
- Transplants are cut with fine scissors from the donor slice and transfer to the host slice with a 20 μ l pipette.

PREPARING MEDIA

Sterile filtered 1x Krebs for post-holding of sections:

1x Krebs	49	ml	
1M Hepes	0.5	ml	
Pen-Strep	0.5	ml	
Gentamicin	0.1	ml	

Medium with serum (MEM):

MEM	44	ml
FCS	5	ml
50% glucose	0.5	ml
100x Pen-Strep	0.5	ml

Serum free Medium (Neurobasal):

Neurobasal	47.5	ml
B-27 suppl.	1	ml
50% glucose	0.5	ml
100x Pen-Strep	0.5	ml
100x Glutamine	0.5	ml

STOCKS

10x Krebs buffer (keeps 1 moth at 4°C). Concentrations are for 1x:

NaCl	(126 mM)	73.6	g	
KCl	(2.5 mM)	1.87	g	
NaH ₂ PO ₄	(1.2 mM)	1.66	g	(monobasic, monohydrate)
MgCl ₂	(1.2 mM)	2.44	g	

CaCl ₂	(2.1 mM)	3.68	g
dH ₂ O		1	l

Gentamicin (CCF)

Hepes (CCF)

MEM with glutamine, α -medium (Gibco)

Neurobasal (Gibco)

50% Glucose (in dH₂O, sterile filtered)

100x Pen-Strep (CCF)

B-27 (Gibco)

100x Glutamine (CCF)

OTHER MATERIAL

Polycarbonate culture membranes, 13 mm diameter, 8 μ m pore size: Costar-110414.