Solutions for Cortical Cell Cultures

(for E17 to P0 cultures)

HBSS (for dissection)
(use within 1 month)

10X HBSS (Gibco #310-4180) 50 ml
1 M Hepes (pH 7.4) 1.25 ml (=2.5mM)
1 M Glucose 15 ml (=6.5 mg/ml=35 mM)
100 mM CaCl2 5 ml (=1mM)
100 mM MgSO4 5 ml (=1mM)
1 M NaHCO3 2 ml (=4mM)

Add sterile dH2O to a total vol. of 500 ml.

Dissociation Medium (DM)
(use within 1 month)

1 M Na2SO4 20.44 ml (or 40.88 mls of 0.5 M Na2SO4) (keep stock at room temp.)
0.5 M K2SO4 15 ml (keep stock at room temp)
1 M MgCl2 1.45 ml
100 mM CaCl2 0.63 ml
1 M Hepes (7.4) 250 µl
1 M Glucose 5 ml
Phenol Red (0.5%) 0.5 ml
0.1N NaOH 0.5 ml

Add sterile dH2O to a total vol. of 250 ml.

[Make fresh enzyme and inhibitor solutions immediately before dissection. For P0 cultures, add Ky/Mg to enzyme and inhibitor solutions]

Enzyme soln.

DM 10 ml
Add cystein-HCl 3.2 mg
Add Papain 200 units
Mix and let dissolve for 15 min. (@ 37 C)
pH w/ 0.1 N NaOH (approx. 6 drops)
or O2/CO2 (pink too basic/ yellow too acidic)
Filter through 0.2 um syringe filter.

Heavy Inhib. soln.

DM (37 C) 6 ml
BSA 60 mg
Trypsin Inhib. 60 mg
Place at 37 C.
Mix and pH w/ 0.1 N NaOH (approx 12 drops)
or O2/CO2 (pink too basic/ yellow too acidic)
Filter through 0.2 um syringe filter.

Light Inhib. soln.

DM (37 C) 9 ml
Heavy Inhib. 1 ml
Filter through 0.2 µm filter.
Place at 37 C.

E17/P0 Serum-Free Media (Neurobasal+B27)

Neurobasal (GIBCO without glutamine) 47.5 mls
B27 supplement (GIBCO) 1 ml
Glutamine (200 mM) 0.5 ml
Pen/Strep (GIBCO#15140-122) 1 ml

Notes:

1. Use 3ml medium per 60 mm plate. Replace half the media every 6 days.

2. Ara-C may be added two days after plating to P0 cultures to prevent glial cell overgrowth.

3. Alternate media:

F12/DMEM media

100 mls

F12 47 mls
DMEM (w/o glutamine) 47 mls
PenStrep 1 ml
Glutamine(200mM) 250µl
Rat Serum 5 mls

L15 medium

L15 (w/o glutamine) 500 mls
1M Glucose 15 mls
1M $NaHCO_3$ 12.9 mls
PenStrep 5 mls

4. Glucose concentrations typically used:

McConnell: 3g/liter (1:100 of 30% Glucose)
Baraban: 5.5 g/liter
Bading: 3.6 g/liter (20 mM Glucose; 1 M Glucose=186 g/liter)