Dissociated Cultures of Cerebellar Neurons

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Protocol

1. **Dissection**

   Can do on counter (i.e., in non-sterile conditions)
   Use P8 rat pups, or P5 mouse pups
   Keep on ice whenever possible
   For each step, do all pups, before moving to next step

   Fill 2 tubes with approx. 3 0ml HHGN (one for dissection; other for cell processing -- keep sterile); keep on ice
   Fill ice tray
   Sterilize tools by soaking in EtOH: large forceps, 2 fine forceps, curved forceps, 1 small and 1 large scissors
   Place TDn, DnB at room temp to thaw

   a. Isolate cerebella (ñCbî)
   b. Cut off head into plate with HHGN
      Hold nose with large forceps
      Cut with scissors thru skin and skull, from side of neck, to top of head, across, and back to neck
      Remove flap of skin/skull from front to back
      Pinch off cerebellum with fine forceps, into HHGN
   c. Remove meninges
   d. Under dissecting scope, pull away using two fine forceps
      Not necessary to remove entirely
   e. Chop each cerebellum into approx 4 pieces (not necessary for mouse, if Cb small)

2. **Processing**

   In tissue culture hood; keep sterile
   Transfer tissue to 15 ml pop-top tube (use 25 ml pipet so opening is large)
   Rinse 3 X 2ml HHGN
   Digest 15 min, room temp, in 5 ml TDn (lower volume OK if doing individual Cb, eg. for mice)
   Remove sup, wash 3 X 3 ml HHGN
   Add 5 ml DnB; transfer to 50 ml tube
   Triturate with 5 ml pipet until homogeneous (20 X)
   Let settle 5 minutes to remove clumps; transfer sup to second tube

   (optional:)
   Re-triturate clumps with 5 ml more DnB (more vigorously; seal pipet tip, triturate approx. 10X)
   Let settle 5 minutes; transfer/pool sup with first sup

   Centr 500 RPM, 3 min, R.T.
   Resusp pellet in 10 ml culture media (should resuspend easily)
   Count live/dead cells: eg.:

Dilute 1/10: 65 ul media (or HHGN) + 25 ul 0.4% trypan blue + 10ul cells [or, dilute 3/4 if low conc cells]; count 10 ul
Expect approx 50% live cells

Dilute cells in media, and seed plates/wells:
24 well: 5 X 105 live cells 0.5 ml 35 mm: 2.5 X 106 1.5 ml 60 mm: 5 X 106 3 ml 100 mm: 1.5 X 107 9 ml note, 24 w: shake plates vigorously left-right, top-bottom, before placing into incubator, to evenly distribute cells don't need to change media after seed (eg., 2 h) grow in 5% CO2, at 37ºC on 1 DIV (approx 24 h), add araC to 10 uM: 24 well: 10 ul 500 uM 35 mm: 30 ul 500 uM [araC: -80ºC box G5 (HD) or E1] 60 mm: 60 ul 500 uM feed with new media (containing araC) on (3)-4 DIV: 1 ml/3 ml per 60 mm 200 ul/500 ul per 24 well [can do when replace media after transfection]

3. **Death Induction**
   On 6 DIV (have also done on 7 DIV) wash 2X with -KI 24 w: 0.5 ml 35 mm: 1.5 ml 60 mm: 2 ml add -KI or +KI

Reagents/Solutions

1. HHGN
2. 500 ml 1X HBSS 50 ml 10X [without Ca, Mg; Gibco/BRL # 14180-020] 2.5 mM Hepes pH 7.3-7.5 1.25 ml 1 M glucose 7 ml 2.5 M (45%) NaHCO3 2 ml 1 M store at 4ºC (approx 2 mo.)
3. DNAase
4. Boehr-Mann #104159 (2,000 u/mg); bovine pancrease, grade II stock: 4,000 u/ml in H2O; sterile filter, store at -80ºC
5. TDn
6. 250 ul DNAase 5 ml HHGN 50 mg trypsin [Worthington # 3703] pH with 0.1 N NaOH sterile filter note, can make up large amount, aliquot to 5 mls, and store at -80ºC (approx 1 mo.) thaw to RT before use
7. DnB
8. 500 ul DNase I 10 ml BME [Sigma #B1522] can make up large amount, aligout to 10 mls, and store at -80ºC thaw to RT before use
9. 3 M KCl
10. May need to autoclave to sterilize; too viscous to filter ?
11. BME+K
12. 500 ml BME [5 mM K] 4.1 ml 3 M KCl (therefore, 24 mM additional, 29 mM K total) [unopened BME bottle: 523 ml on average]
13. Calf serum
14. Hyclone# A2151, need to heat inactivate sterile filter; aliquot to 50 ml and 10.5 ml; store at -20ºC
15. Culture media (ñCbC Medî)
16. 100 ml BME+K 10 ml calf serum 1 ml 0.2 M glutamine [use fresh tube] 1 ml Penn-Strep when refeed, add araC to 10 uM: 40 ul 25 mM per 100 ml final [K]= 26 mM

17. -KI
18. 100 ml BME [5 mM K] 1 ml 0.2 M glutamine 1 ml Penn-Strep 40 ul 25 mM araC [10 uM final]
19. +KI
20. Per 10 ml: 10 ml -KI 25 ul 2 mg/ml insulin [5 ug/ml final] 67 ul 3M KCl [20 mM additional, final = 25 mM] sterile filter (if insulin stock is unsterile)

Notes

Usually use P8 Long-Evans rats; have also used P5-9 successfully; P5/6 rats yielded considerably fewer cells, and a relatively higher number of glia; published protocols also use Sprague-Dawley and Wistar rats yield of cells is approx 1.5 X 107 cells per P8 rat original protocols call for fetal bovine serum; however, calf serum was used and seems satisfactory insulin is used for survival, rather than IGF-1 of ref 1, due to cost although ref 1 reports survival in KCl or IGF (in place of serum, at 6DIV), mouse Cb cells survive better with both present (for rat Cb cells, KCl alone allowed full survival) without media change, in CbC media (serum + KCl), rat Cb cells live only about 7-9 days; with 1 change (4DIV), live approx 12 d.

References

1. DÍMello *et al*. (1993) PNAS 90, p. 10989.
2. Galli *et al.* (1995) J. Neurosci. 15, p. 1172.