

PREPARATION OF GLIAL CULTURES

1. Kill neonatal rat pups by cervical dislocation. preferred age is on day of birth; animals can be up to 2 to 3 days old. Sterilize skin with 70% EtOH.
2. Decapitate pups, then, in laminar flow hood, remove brains, and dissect out the cerebral hemispheres in BSS.
3. Using a dissecting microscope, carefully strip away all of the meninges.
4. Place the hemispheres in a petri dish in BSS and chop as finely as possible with scissors.
5. Transfer the minced tissue to a small trypsinizing flask in a final volume of 13.5 ml of BSS. Add 1.5 ml of trypsin (2.5% stock solution) and 1.5 ml of DNase (1% stock solution).
6. Incubate at 37°C for 15 min., stirring continuously at a slow speed.
7. Pour off the supernatant (containing the dissociated cells), leaving undissociated tissue behind. Pour the supernatant through sterile nylon mesh (72um) to remove any chunks of tissue and collect in a small beaker. Dilute with an equal volume of 10% horse serum in (GLIAL) MEM (to inhibit the trypsin).
8. Bring the volume in the flask (containing tissue still undissociated) back to 15 ml. by adding 0.25% trypsin. Heat and stir for an additional 10 min.

Preparation of Glial Cultures

9. If a substantial amount of tissue remains undissociated, pipet this gently to dissociate it further. Then pour the supernatant through the mesh, add to previous supernatant, and add 10% horse serum. Rinsing the flask with BSS will give a few more cells.
10. Centrifuge for 5 minutes (setting 6) to pellet the cells. Discard the supernatant (containing the trypsin), and resuspend the pellets in 2 ml per centrifuge tube of 10% horse serum. Combine the resuspended pellets with MEM + 10% horse serum to a final volume of 100 ml.
11. Count cells with a hemacytometer. Yield should be on the order of 15 million cells per brain. Dilute cells to desired concentration in 10% HS in MEM, and plate into 60 mm tissue culture dishes (Falcon* # 3002) at 4 ml per dish. Our standard density is 100,000 cells per ml; when it is necessary to obtain confluent monolayers as quickly as possible, we increase this to 300,000 cells per ml.
12. Glial cultures are fed twice per week using MEM with 10% horse serum.

Materials Needed for Culturing Glial Cells

1. At least 8 neonatal rat pups (preferably born the day of the experiment).
2. 5 sterile large petri dishes

Out of hood:

1 for collecting pups

1 for collecting pup heads

1 for collecting pup bodies

In hood:

1 top for skull instruments

1 bottom for BSS & cutting out brains

1 for meninges instruments

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3. Sterile surgical instruments
 - A. small scissors (for cutting up brains)
 - B. 2 # 5 forceps (with fine tips for removing meninges)
 - C. small scissors & 2 microforceps (for removing brains)
 - D. large scissors “rat-tooth” forceps (for removing heads)
4. 1 small petri or tissue culture dish with 7 ml warm BSS to collect and cut hemisphere in.
5. Sterile trypsinizing flask & magnetic stir bar
6. 2 Sterile beakers (150 ml or 200 ml)
 - 1 for transferring cut hemispheres to trypsinizing flask
 - 1 for collecting and neutralizing trypsinizing cells
7. 3 30 or 60 ml sterile syringes with nylon mesh (73 um)
8. 1.5 ml 2.5% trypsin in BSS
9. 1.5 ml 10X DNase
10. 1 100 ml bottle BSS (Ca, Mg-free) (warmed to 37°C)
11. Sterile tissue culture dishes
12. 10 sterile paraffin dishes
13. 2 500 ml bottles MEM + 10% HS (GLIAL) warmed at 37°C.