

MEI Protocol

Astroglial cell culture

By Yanmei Tao

- 1, 2-3 P3-P5 mouse pups. (Cells from embryo to P1 mouse grow very slowly.) Rinse the anesthetized animals few seconds with 70% alcohol, and then quickly transfer them into sterilized 1XPBS. Rinse animals with plenty 1XPBS twice.
- 2, Isolate cerebral hemispheres, and get rid of meninges with fine tip forceps. Mince to small pieces. All these process should be done in Ca/Mg free HBSS.
- 3, Trypsinize with 0.25% Trypsin at 37 C for 15 mins.
- 4, Pipette up and down for 10 times to dissociate cells. (optional: if viscous DNA is visible, add DNase into cell suspension, pipette 5 times more.)
- 6, Centrifuge 1,000g for 5 mins. Get rid of supernatant, resuspend cells with DMEM+10%FBS (Culture medium. MEM+10%Horse serum also works.) Pass through a 70um cell strainer. Count cell number by using a hemacytometer.
- 7, Dillute the cell suspension to about 100,000cells/ml. Plate into 60mm dish (5ml/dish), 100mm dish (10ml/dish).
- 8, Change medium twice a week. 10-14 days cells will reach confluence.