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# Primary Neuron-glia Culture from Rat Cortex as a Model to Study Neuroinflammation in CNS Injuries or Diseases

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**[Abstract]** Primary neuron-glia cultures are commonly used *in vitro* model for neurobiological studies. Here, we provide a protocol for the isolation and culture of neuron-glial cells from cortical tissues of 1-day-old neonatal Sprague-Dawley pups. The procedure makes available an easier way to obtain the neuron and glia. In this culture system, neuron-glia cultures consisted of approximately 37% neurons, 51% astrocytes, 7% microglia, and a small percentage (<5%) of other cells after fourteen days *in vitro*. Primary neuron-glia cultures is a simplified *in vitro* model for studies focusing on interactions between neurons and glia cells. Activated glial cells, mainly astrocytes and microglia, are histopathological hallmarks of acute injury of the central nervous system (CNS) or chronic neurologic diseases (Hirsch and Hunot, 2009; Lee *et al*., 2009; Minghetti, 2005). Inflammatory mediators (*e.g.,* nitric oxide, reactive oxygen species, proinflammatory cytokines, and chemokines) released by activated glia can directly or indirectly cause neuronal damage or neurodegeneration. Neuroinflammation is a common mechanism of various neurological diseases leading to neurodegeneration. The advantages of neuron-glia cultures are that: (1) Cultured cells can bypass complicated physiological interactions (such as leukocyte infiltration, blood-brain barrier, reflex or other systemic regulation) *in vivo* to allow direct observation of neuroinflammation caused by various CNS insults (hypoxia, ischemia, trauma. infection, neurotoxins, chronic stress or diseases); (2) Unlike cell lines that are mostly derived from tumor cells, primary cultured neuron-glia system is closer to the cell population ratio *in vivo* and can mimic the *in situ* microenvironment; and (3) Cultures can be prepared from various brain regions (*e.g.,* cortex, hippocampus, mesencephalon…*etc*) and allow an opportunity to examine the regional difference in the susceptibility to neurodegeneration following neuroinflammation caused by various CNS insults (Kim *et al*., 2000). The following protocol is an example for primary rat cortical neuron-glia culture preparation (Huang *et al*., 2015; Huang *et al*., 2014; Huang *et al*., 2012; Huang *et al*., 2009).

 

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## Materials and Reagents

1. Tissue culture dishes (60 x 15 mm) (Sigma-Aldrich, catalog number: P5237)
2. Tissue culture dishes (100 x 20 mm) (Nunc, catalog number: 172958)
3. Screw cap centrifuge tube (50 ml) (Sigma-Aldrich, catalog number: BR114821)
4. 24-well plates (Sigma-Aldrich, catalog number: CLS3527)
5. Pipette tips (10 µl, 200 µl and 1,000 µl) (Shineteh instruments co ltd., catalog number: PT4-W10, PT1-Y200 and PT5-W10)
6. Neonatal Sprague-Dawley pups (1-day-old)
7. Trypan blue (Thermo Fisher Scientific, Gibco™, catalog number: 15250061)
8. Hanks' Balanced Salt solution (HBSS) (Sigma-Aldrich, catalog number: 55021C)
9. Sodium bicarbonate (Sigma-Aldrich, catalog number: S5761)
10. Pyruvate (Sigma-Aldrich, catalog number: P2256)
11. HEPES (Sigma-Aldrich, catalog number: H3375)
12. Bovine Serum Albumins (BSA) (Sigma-Aldrich, catalog number: A9418)
13. DMED powder (Thermo Fisher Scientific, Gibco™, catalog number: 12100-046)
14. DMED/F-12, HEPES, no phenol red (Thermo Fisher Scientific, Gibco™, catalog number: 11039-021)
15. Penicilline/Stretomycin (100x) (Thermo Fisher Scientific, Gibco™, catalog number: 15140-122)
16. 100 mM Sodium pyruvate solution (100x) (Thermo Fisher Scientific, Gibco™, catalog number: 11360-070)
17. MEM non-essential amino acids solution (100x) (Thermo Fisher Scientific, Gibco™, catalog number: 11140-050)
18. Fetal bovine serum (FBS) (NQBB, catalog number: A6806-11)
19. Hank's solution (see Recipes)
20. Dulbecco's modified Eagle's medium (DMEM) (see Recipes)
21. Serum-free medium (100 ml) (see Recipes)

## Equipment

1. 37 °C, 5% $CO_2$ incubator (Water Jacketed Laboratory $CO_2$ Incubator)
2. Stereo microscope (Shineteh instruments co., catalog number: IH1-ZM150A)
3. Biological Inverted microscope (OLYMPUS CORPORATION, model: IX71)
4. Mini micro centrifuge (Shineteh instruments co., catalog number: IC-MINIMAX)
5. Centrifuge (Hermle, catalog number: Hermle Z232K)
6. Water bath (Bioman Scientific Co Ltd, catalog number: SWB-20-1)
7. Counting chamber (Shineteh instruments co., catalog number:PT14-901001)
8. Operating scissors STR (Shineteh instruments co., catalog number: ST-014)
9. Iris scissors STR (Shineteh instruments co., catalog number: ST-S009)

 

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10. Dressing forceps (Shineteh instruments co., catalog number: ST-D114)
11. Iris forceps (Shineteh instruments co., catalog number: ST-I510)
12. Tweezers forceps (Shineteh instruments co ltd., catalog number: ST-NO5)
13. Pipetman (Gilson, catalog number: P10, P200 and P1000)
14. Pipet-aid (Thomas Scientific, Flacon™, catalog number: 0410C04)
15. Pipets (10 ml) (Tseng Hsiang Life Science ltd., catalog number: SP-1-C)
16. Ice bucket (Shineteh instruments co ltd., catalog number: PA8-4)

## Procedure

1. Primary neuron-glia cultures are prepared from cortical tissues of 1-day-old neonatal Sprague-Dawley pups.
2. After the rats are sacrificed, their brains are quickly removed aseptically. Under the stereo surgical microscope, the blood vessels and meninges are removed by tweezers.
3. Cerebral cortices (6~8 pups cerebral cortices/tube) are dissected under sterile conditions and kept on ice in conical tube containing 10 ml of Hank's solution (without $Ca^{2+}$ or $Mg^{2+}$).
4. Remove Hank's solution and add 15 ml of warm (37 °C) Dulbecco's modified Eagle's media (DMEM) containing 10% heat-inactivated FBS to 50 ml conical tube.
5. Cortical cells are dissociated by trituration using a 10 ml pipette (about 15 times). Cells are centrifuged at 4 °C (1,500 *x g*) for 5 min to pellet cells.
6. After centrifugation, cells are re-suspended in 10 ml of DMEM containing 10% heat-inactivated FBS.
7. Dilute cells for counting by adding 20 µl of cells to 180 µl of culture medium (10x dilution).
8. Add 10 µl of the above cell dilution to a hemocytometer. Cells are counted in the 1 mm center square.
9. Cell density is determined by the average of the 5 randomly selected squares. To calculate the total # of cells/ml = (the average count per square) x (the dilution factor) x ($10^5$/ml).
10. Dilute the 10 ml stock to make a final concentration of 5 x $10^5$ cells/ml.
11. To each well of 24-well culture plates 5 x $10^5$ cells are seeded in 0.5 ml of culture medium.
12. The cultures are incubated at 37 °C in a humidified atmosphere of 5% $CO_2$ and 95% air.
13. Culture medium is replenished five days after plating, and is changed every three days thereafter.
14. The neuron-glial cells become confluent 10-12 days after plating.
15. Fourteen days *in vitro* (DIV14), the cultures are used for experiment. The cell

 

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composition is determined by immunostaining, followed by cell counting. The neuron-glia cultures consisted of approximately 37% ± 0.8% neurons, 51% ± 1.5% astrocytes, and 7% ± 7.7% microglia (Figure 1). In addition, cultures also consist of a small percentage (<5%) of other cells including oligodendrocytes, fibroblast and endothelial cells (data not shown).

16. The characterization of neuronal phenotypes is shown in Figure 2.

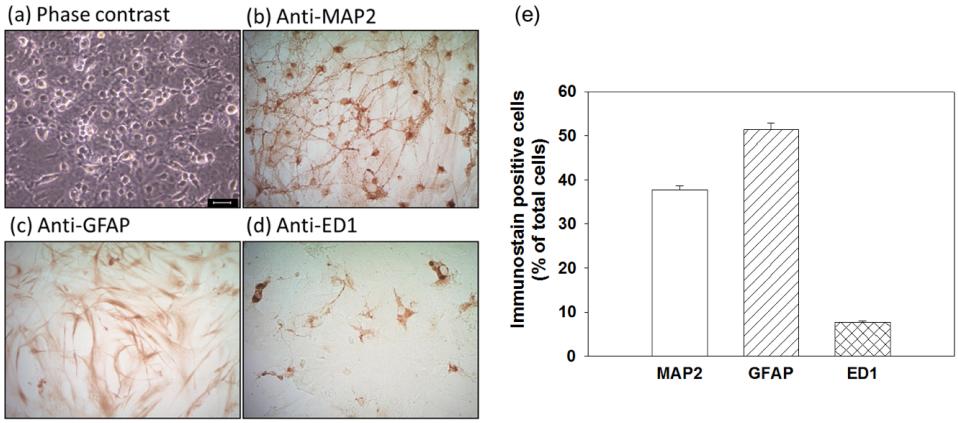


**Figure 1. The composition of cell types in primary cortical neuron-glia cultures.** Phase contrast photomicrograph of the primary cortical neuron-glia cultures *in vitro* (DIV14) (a), and representative immunocytochemically-stained photomicrographs using antibodies against (neuronal) dendritic marker microtubule associated protein 2 (MAP2) (b), astrocytic marker glial fibrillary acidic protein (GFAP) (c) or pan-macrophage lysosomal antigen (ED1) as a phagocytic marker for activated microglia (d). Percentage of immunostain positive cells of neurons ($MAP2^+$), astrocytes ($GFAP^+$) and activated microglia ($ED1^+$) cells in cultures (e). Scale bar = 25 μm

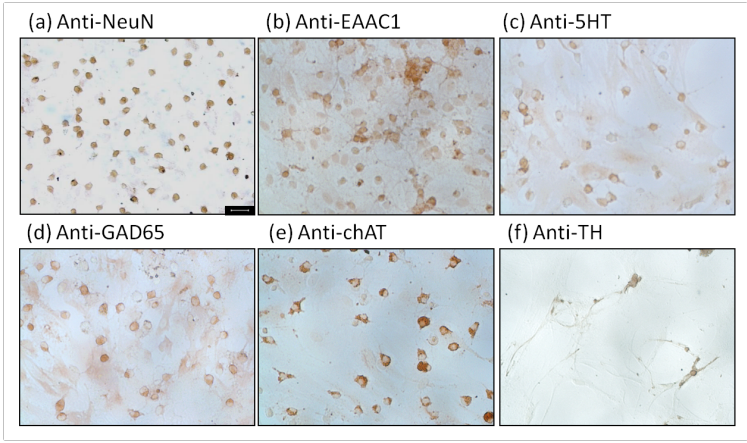


**Figure 2. Characterization of neuronal phenotype by immunocytochemical staining.** Cultures were stained using antibodies against (a) neuronal marker

 

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neuronal nuclei antigen (NeuN) on 14 days *in vitro* (DIV 14), (b) excitatory amino acid carrier 1 (EAAC1) for glutamatergic neurons, (c) 5-hydroxytryptamine (5HT) for serotonergic neurons, (d) glutamate decarboxylase 65 (GAD 65) for GABAergic neurons, (e) choline acetyltransferase (ChAT) for cholinergic neurons, and (f) tyrosine hydroxylase (TH) for catecholaminergic neurons. Scale bar = 25 μm

## Recipes

1. Hank's solution (1,000 ml)

| Reagents | Volume |
|---|---|
| Hanks' balanced salt solution | 1,000 ml |
| Sodium bicarbonate | 0.35 g |
| Pyruvate | 0.11 g |
| HEPES | 4.76 g |
| Bovine serum albumins (BSA) | 3 g |

2. 10% fetal bovine serum (FBS) / Dulbecco's modified Eagle's medium (DMEM) (1,000 ml)

| Reagents | Volume |
|---|---|
| DMED powder | 1 package |
| Sodium bicarbonate | 2.2 g |
| Pyruvate | 0.11 g |
| HEPES | 4.76 g |
| FBS | 110 ml |

3. Serum-free medium (100 ml) (drug treatment only)

| Reagents | Volume |
|---|---|
| DMED/F-12, HEPES, no phenol red | 97 ml |
| 100x Penicilline/Stretomycin | 1ml |
| 100x sodium pyruvate solution (100 mM) | 1 ml |
| 100x MEM non-essential amino acids solution | 1 ml |

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