

## **Astrocyte Cell Culture**

### **Preparation of flasks:**

1. Coat T75 flask(s) with 1 mg/ml of PureCol (Collagen) overnight
2. Remove solution, rinse flasks with sterile ddH<sub>2</sub>O, set the flasks upright and allow to dry in culture hood for 2 hr

### **Dissection:**

1. Dissect P1-P3 pups: Remove brainstem, cerebellum and diencephalons in cold dissection buffer. Peel off meninges and transfer cortex to a 50 ml tube on ice, which contains 20 ml of cold dissection buffer. (Dissect 2 pups for  $2 \times 10^6$  cells/flask).
2. Carefully pour tissue into a 10 cm dish and gently mince tissue with sterile scissors or razor blade.
3. Transfer tissue to back to 50 ml tube and add 5 ml 1X trypsin and 50  $\mu$ L DNase for 25 min at 37°C. Swirl tube every 5 min.
4. Wash the cortices with Glial Medium twice.
5. Dissociate the tissue by gently triturating the cortices through a 5 ml or 2 ml pipette, followed by a fire-polished Pasteur pipette (3 X 3 triturations). Each time fill pipette with dissociated cells and transfer supernatant to a fresh tube.
6. Dilute cell suspension to 10 ml of Glial Medium, and pass through a 40  $\mu$ M cell strainer.
7. Spin down the cells at 1700 rpm for 5 min.
8. Re-suspend the cells with 10 ml of Glial Medium, and count.
9. Seed  $2 \times 10^6$  cells/flask in 15 ml Glial medium.  
\*\*\*\*( $2.0 \times 10^6$  cells/flask =  $1.33 \times 10^5$  cells/ml =  $2.67 \times 10^4$  cells/cm<sup>2</sup>)\*\*\*\*
10. Change the medium each of the next two days by aspirating the medium, and then adding back 15 ml of fresh Glial Medium.

### **Passaging/Freezing:**

Glial cells should be confluent within 6-7 days. You should have at least  $2 \times 10^6$  cells per flask. When you passage the cells, reseed at  $3 \times 10^5$  cells/75 cm<sup>2</sup> flask.

\*\*\*\*( $3 \times 10^5$  cells/flask =  $2 \times 10^4$  cells/ml =  $4 \times 10^3$  cells/cm<sup>2</sup>)\*\*\*\*

You need to prepare a fresh culture every three weeks.

### **Preparation of 6 cm dishes (Prepare the dishes on Thurs/Fri, then do the hippocampal dissection on the next Tues/Wed):**

**Thursday:** Coat the dishes with 1 mg/ml of Collagen; wash with sterile ddH<sub>2</sub>O & allow to dry in the culture hood for 30 minutes.

#### **Friday:**

1. Wash the glial cells with PBS once.
2. Add 3-5 ml of trypsin to the culture flask; incubate at 37°C for 5 minutes.
3. Add 5-7 ml of Glial Medium to the culture flask, and then transfer the cells to a 50 ml conical tube.
4. Spin down the cells @ 1700 rpm for 5 minutes.
5. Remove the supernatant, then add 10 ml of Glia medium to re-suspend the cells
6. Seed  $7.5 \times 10^4$  cells/60mm dish in 6 ml of Glial medium  
\*\*\*\*( $7.5 \times 10^4$  cells/dish =  $1.25 \times 10^4$  cells/ml =  $3.6 \times 10^3$  cells/cm<sup>2</sup>)\*\*\*\*

### **Dissection Medium**

50 ml 10X HBSS (w/o Ca and Mg, Gibco 14185-052)

5 ml Pen-Strep (Gibco 15140-122)

5 ml Na Pyruvate (Gibco 11360-070)

\*\*\*\*(CAN ADD 1.15 ml for final pyruvate concentration of 0.23 mM)\*\*\*\*  
10 ml HEPES (Gibco 15630-080)  
5 ml (25mM) Glucose (Sigma G-8769 2.5M 45%)  
\*\*\*\*(CAN OMIT THIS and glucose concentration will be 5 mM final)\*\*\*\*  
435 ml ddH<sub>2</sub>O

**Glial Culture Medium**

430 ml DMEM  
50 ml FBS  
5 ml Pen/strep  
5 ml Na Pyruvate  
\*\*\*\*(Can add 1.15 ml for final pyruvate concentration of 0.23 mM)\*\*\*\*  
10 ml Gluta-Max (Invitrogen 35050-061)

**Product Ordering Information:**

Vitrogen (PureCol) – Type I Bovine Collagen (#5409)  
Inamed Biomaterials  
800-883-8220