Glia primary cultures

NOTE: Because this is a primary culture always use Quarantine incubator and do NOT USE the suction system before the Mycoplasma test is done!

Dissection

This is done 3 weeks before plating
Use P2 pups

Prepare in advance

- Glia medium (50mL FBS, 5mL P/S, 15mL D-Glucose (3gram of D-Glucose in 15ml of Alpha Mem) fill to 500mL with Alpha Mem)
- 2 tubes of cold Glia Medium (2 tubes per 2 brains (1wash 1 collect))
- 1 tube of warm Glia medium
- Dissection tools
- Box with ice

In the hood prepare:

- One 15cm dish per 2 brains + 25ml warm glia medium
- 10mL syringe + 20G needle
- One 50ml tube per brain

Procedure

1. Collect the head of the P2 pup in the cold glia medium
2. Dissect brain out of skull in petri dish with ice cold Glia medium
3. Pass the brains to new petri dish with clean cold glia medium.
4. Repeat steps 2 and 3 to every brain.
5. In the hood: pipet 5ml of glia medium in each 50mL falcon
6. Suck 1 brain with needle and place it in 50mL Falcon
7. Do this for every brain
8. Resuspend the brain pieces with the needle until there are very small pieces left (around 2-3 times)
9. Fill the 15cm dish with 25ml of warm glia medium
10. Pipet the brain pieces in the 15cm dish, Plate 2 brains per dish
11. Rinse the 50mL falcons with 5ml clean Glia medium and add to the 15cm dish
12. Place in quarantine incubator
13. Change medium 24hrs later
14. Change medium every 3-4 days

Glia protocol, 22-2-19, V1

Expansion (1x week, at least 2x before ready for plating)

1 dish will be divided over 3 new dishes

1- Wash 2x with PBS
2- Add 2,5 mL trypsin EDTA for 5minutes at 37C
3- Add 7,5 mL glia medium and collect to 50mL Falcon
4- Rinse dishes with 10mL and collect
5- Centrifuge for 4 minutes at 200 rcf
6- Remove supernatant and add 5mL of glia medium per new dish
7- Plate 5 mL per dish
8- Change medium every 3-4 days

Plating the GLIA (eg.12 well plates, see motor neuron differentiation protocol)

Glia cells are co-cultured with neurons in the final stages of maturation (see motor neuron differentiation protocol)

Before plating, make 12-well plates with 3 droplets of paraffin in the wells. UV the dishes before using them.

Do steps 1 to 4 of the **glia expension** protocol

Collect 15uL to Rosenthal to count before step 5

X=counted glia

(X /4) x 5000 = cells/mL cells/mL x iV = total cells

Total cells	
36000 cells	1ml

Total cells x 1ml / 36.000 = mL to re-suspend the total cells in.

Remove supernatant and re-suspend in appropriate volume for plating

Plate 36000 cells per well