**Isolation and culture of cortical neurons from mouse brain**

**A. (DAY 0) Preparation of medium and plates on which the neurons will be cultured**

* Prepare the **media** for the isolation and culture of the neurons the day before the isolation
  + Equilibrate the media (37°C, 5% CO2) in a T75/T150 flask with filter top
* Coat the plates for culture with 50 μg/ml **poly-L-ornithine** 
  + Put the PDL solution (in **MilliQ**) in the plates for 2h (incubator ON)
  + Wash with PBS (2x)
  + Let the plates air dry for at least 2h (store dry in fridge)

**B. (DAY 1) Isolation of cortical neurons**

1. Pre-warm **HBSS/Hepes** to 37°C and put ±10 ml in a petridish to collect embryos.
2. Fill one of the 35 cm dishes with 2.5 ml HBSS (to collect cortices)
3. Thaw **1 ml** **trypsin (10x) Solution** to room temperature
4. Kill a P0 mouse by decapitation
5. Transfer the heads to the petri dish containing pre-warmed HBSS/Hepes.
6. With a new set, stab the forceps into the forehead (eyes).
7. With another curved forceps, stab from the top (start just above the eyes) and remove the skull using a lateral (outward) movement (one hemisphere at the time). Bend the skull away – make sure there is no more bone left before starting to remove the brain.
8. Scoop up the brain using the curved forceps
9. Put the brain with the ventral side facing up into a dish containing HBSS/Hepes.
10. Cortices are removed under a dissecting microscope: Remove the 2 hemispheres from the diencephalon and the brain stem (cut & pull).
11. Put the hemispheres with the internal side facing up.
12. Grasp the meninges and pull it toward and around the olfactory bulb and continue until the meninges are pulled away from the whole cortex and you reach the thalamus. Try not to break the meninges!
13. Remove thalamus and hippocampus by cutting along the convex outer side (you can cut using 2 straight thin forceps).
14. Remove the striatum by cutting between the cortex and the striatum
15. Now only the cortex remains (bean shape like structure)
16. Transfer them into the dish with pre-warmed HBSS/Hepes.
17. After the cortices are dissected, transfer them into a 50 ml tube with a sterile polypet (met stukje afgeknipt).
18. Remove the HBSS/hepes as much as possible.
19. Add 9 ml HBSS/Hepes (pre-warmed)
20. Add 1 ml of Trypsin (10x).
21. Incubate for 15 minutes (no longer) at 37°C.
    * Prepare 3 falcon tubes with 5 ml **plating medium (MEM-horse)**
22. Add an equal amount of plating medium (let tissue sink).
23. Transfer the tissue to the first tube with plating medium (let tissue sink)
    * Repeat this washing step an additional 2 times
24. Mechanically dissociate the cortical tissue:
    * dissociate the tissue by pipetting 10-15 times up and down using a blue micropipet
25. let small clumps sink and transfer only the cell suspension to 15 ml tube
    * Transfer via a cell strainer (70 μm)
    * Rinse the filter with some medium
26. Spin down the cells by centrifuging them at 300g for 6 minutes
27. Dispose supernatant and resuspend in ± 5 ml MEM-horse
28. Count the cells
    * In 96-well plate: 80 000cells/well🡪100ul
    * In special glasses for staining: 35 000cells/well 🡪250ul

**C. Culturing the cortical neurons**

1. Plate the cells at the desired density (na het uitplaten mag het plating medium weg)
2. 4 hours (up to 24h maximum) later, after the cells are nicely attached, take off the plating medium completely and replace gently with the pre-equilibrated **B27/L-glutamine neurobasal medium**
3. Keep in culture at 37°C and 5% O2
   * Medium change every 3-4 days, only *half the medium per time!!*

**Products and preparation of the media**

**HBSS/Hepes** (make 100 ml per isolation)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Product** | **Supplier** | **[Stock ]** | **[Working]** | **Total 100 ml** |
| HBSS (-Ca, -Mg, -Phenol) | Gibco (14175-095) | - | - | 98 ml |
| Hepes buffer solution | Gibco (15630-049) | 1 M | 7 mM | 0.7 ml |
| Pen/Strep | Gibco (15140-122) | 10.000 U/ml | 100 U/ml | 1 ml |

* Filter through a 0.22um filter and store at 4°C

**MEM-Horse medium (plating medium)** (make 100 ml per isolation)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Product** | **Supplier** | **[Stock ]** | **[Working]** | **Total 500 ml** |
| MEM (+Earle’s) | Gibco (31095-029) | - | - | 435 ml |
| Horse serum (heat inact.) | Gibco (26050-088) | - | 10% | 50 ml |
| 20% glucose | - | 20% | 0.6% | 15 ml |
| Pen/Strep | Gibco (15140-122) | 10.000 U/ml | 100 U/ml | 5 ml |

* Filter through a 0.22um filter and store at 4°C
* Let equilibrate in T75/T150 flask with filter top (37°C, 5% CO2)

**Neurobasal medium (culture medium; 100ml)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Product** | **Supplier** | **[Stock ]** | **[Working]** | **Total 500 ml** |
| Neurobasal medium (**-** L-glut) | Gibco (21103-049) | - | - | 485 ml |
| B27 (+ VitA) | Gibco (17504-044) | 50x | 1x | 10 ml |
| L-glut | Sigma (G7513) | 200 mM | 2 mM | 5 ml |
| Pen/Strep | Gibco (15140-122) | 10.000 U/ml | 100 U/ml | 5 ml |

* Filter through a 0.22um filter
* Let equilibrate in T75/T150 flask with filter top (37°C, 5% CO2)

**20% glucose**

* Dissolve 10 g glucose in 50 ml milliQ water using a magnetic stirrer
* Filter through a 0.22um filter and store at 4°C

**Poly-d-lysine**

* Corning, ref nr. 354210

**Glass Pasteur pipettes**

* Disposable glass Pasteur pipettes, 230 mm, Sigma

**Trypsin-EDTA, 0.5% (10x) (- phenol red)**

* Product number: 15400-054, Company: Gibco

**Dissection tools**

* Large forceps and large scissors (dissection of the uterus)
* Small scissors (dissection of foetus and decapitation)
* Forceps nr.5 (x2)
* Curved forceps