NeuroMag Application Note

Primary neurons preparation from rat hippocampi

A- Solution preparation

1- **Dissection solution**: HBSS (without calcium and magnesium) containing 0.25% D-Glucose, keep at 4°C. Prepare fresh solution each time.
2- **Culture medium**: MEM supplemented with 10% Nu serum, 15mM HEPES pH 7.2, 0.45% glucose, 1mM sodium pyruvate, 2mM L-glutamine, 10 IU/ml penicillin-streptomycin.
3- **Culture feeding medium**: MEM supplemented with 2% B27, 15mM HEPES, 0.45% glucose, 1mM sodium pyruvate, 2mM glutamine.

B- Tissue culture vessel preparation

1- Dissolve Poly-L-lysine (Sigma P-1520) at 0.1 mg/mL in water (aliquot and store at -20°C).
2- Cover the coverslip or dish with the Poly-L-lysine solution.
3- Incubate over-night at 37°C.
4- Rinse twice in water.
5- Let dry under a sterile laminar hood.

C- Cell preparation

Prepared cells from (E)18-19 hippocampal neurons. Use glia-neuron co-culture using defined media and not Banker type cultures nor glia feeder layer. NeuroMag reagent is also suitable with neurobasal media.

1- Rinse hippocampi twice in cooled (0°C, max 2-3°C) dissection solution.
2- Dissect hippocampi free of meninges in cooled (2-3°C) dissection solution.
3- Incubate hippocampi solution with trypsin solution (at 0.25 % final concentration) for 15 min at 37°C in one 15 mL Falcon tube. 10 to 20 hippocampi are generally used in 5 ml of solution in 15 ml falcon tube.
4- Stop the trypsin action by washing two times with HBSS or MEM.
5- Resuspend in a final volume of 1 to 3 mL of MEM containing 10 % fetal calf serum.
6- Triturate tissue using a P1000 micropipette (10 times).
7- Wait 1-2 minutes until non-dissociated tissue goes to the bottom of the tube. Transfer dissociated cells into new tube, add 2 mL of HBSS to the remaining non-dissociated clusters and push it (5-10 times) thought Pasteur pipette until complete dissociation of tissue. Transfer the dissociated cells to the tube containing the first dissociated cells.
8- Count the number of cells.
9- Dilute the dissociated neurons to the desired concentration with culture medium. Plate the cells to the polylysine coated dish or coverslip (see the table 1 below for the suggested cell amount). We recommend optimizing the number of cells relative to the dish/coverslip used. For example, 2mL of cells at a density of 300 000 or 400 000 cells per mL can be plated in 35 mm dish. Cell density will also vary according to the desired time point of transfection (immature or mature).

Tissue Culture Dish	Primary neurons
96 well	-
24 well	0.5 – 1.5 x 10^5
6 well	4 – 8 x 10^5
60 mm dish	8 – 18 x 10^5

Table1: Recommended Cell number

10- Grow the cells in 5% CO2 and 37°C in the absence of a glial feeder cell layer in 10% Nu tissue culture medium until the desired time point for transfection.
11- From 10 DIV in culture, change 50 % of old medium with fresh culture feeding medium every 3 days and the day before Magnetofection.

Note that optimal neuronal growth prior to Magnetofection is critical. It depends on the addition of B27 in the culture medium and on the cell density.

Important Observations:

- Hippocampi removed from the brains must be dissected free of the meninges in cooled HBSS without Ca^{2+} and Mg^{2+} and incubated with 0.25% trypsin for 15 min at 37°C. It is essential to remove all meninges from the hippocampi and to respect the time of incubation for the trypsin treatment.
- The temperature of dissection solution is critical; it should not be warmer than 2-3°C.
- Triturate tissue using fire polished Pasteur pipette: 10 times.
- Wait 1-2 minutes until non-dissociated tissue goes to the bottom of the tube. Transfer dissociated cells into new tube, add 2 mL of HBSS to the remaining non-dissociated clusters and push it (5-10 times) thought Pasteur pipette until complete dissociation of tissue. Transfer dissociated cells into the tube with first portion of the neurons.
- Test different concentrations of cells in culture dish.
- Culture medium composition is critical (see section A.2 above)
- The batch of Nu-serum might vary and so it is essential to test different Nu-serum concentrations (10-20%).
- On day 10 of culture, 50% of the medium must be changed with fresh culture feeding medium
- If you need good culture for electrophysiology and transfections, never use AraC.

In addition, you can find some important tips and tricks in the publication of ***Buerli T.*, et al**., "*Efficient transfection of DNA or shRNA vectors into neurons using Magnetofection*" ***Nature Protocols* 2, 3090–3101 (2007).**