NEUROMICS

Hippocampal Neurons Protocol

Courtesy of Emily McMains (Gleason Lab), LSU.

First Batch

We received the hippocampi on 3/10/09. I didn't have time to deal with the tissue until 3/20 and it was stored at 4ºC until then.

1. The day before I made the cultures, I prepared some 35mm dishes by treating them with poly-ornithine (we always have used this instead of poly-lysine) overnight.

2. The next day, I first incubated 4mg papain (from Worthington) in .067mM betamercaptoethanol, 1.1mM EDTA, and 5.5mM cysteine HCl for 30 minutes at room temp per the supplier's instructions.

3. While this was happening, I washed the poly-ornithine solution off of the culture dishes and placed them under UV illumination to sterilize them for 40 minutes. I then made up the enzyme solution (4mg papain in Hibernate Ca) and filtered it with a .2um filter. I removed the tissue media and set it aside and replaced it with the enzyme solution, incubating this in 5%CO2/95%O2 for 30 minutes.

4. After the enzyme treatment, I triturated the cells in the papain solution and then took one half of the volume of the resulting cell suspension and put it in a 15ml centrifuge in the refrigerator where I stored it until I did the second batch of cultures on 3/25.

5. For the first batch, I took the remaining half volume and spun it down at 1500rpm for 2min. I then removed the supernatant (papain solution) and added the 2ml of tissue media that I had set aside earlier. I triturate again to produce a cell suspension, spun it down at 2000rpm for 2 minutes (in retrospect I think I probably could have eliminated the previous step and just removed the papain and resuspended in neurobasal), removed the tissue media supernatant and replaced with 1ml of the Neurobasal provided.

6. I then did the cell count following your protocol and diluted the cell suspension accordingly. I diluted some with the provided neurobasal media and some with our Neurobasal media (just Neurobasal + 1ml penicillin(10mg/ml)-streptomycin (10,000 units)-glutamine(200mM) solution and 1%B27). Realized later that our Neurobasal didn't have glutamate which might explain why those dishes didn't look quite as good. The pictures I'm attaching of the first batch were taken after four days in culture.

Second Batch

1. I took the remaining half of cell suspension that I had saved at 4ºC in papain solution for five days (15 days since the tissue was originally received) and spun it twice for two minutes at 2000rpm, triturated the solution, and then spun it again for one minute at 3000rpm (I had to do all these spins to get a well formed pellet that wouldn't get pipeted out with the supernatant. In retrospect, I probably should have started by triturating and spinning at 3000rpm for 1 minute.)

2. I then removed the supernatant and resuspended with 1ml of the provided Neurobasal. I did a cell count and ended up with many fewer cells in the hemocytometer than I did for the first batch.

3. I diluted the cell suspension accordingly. I ran out of the provided Neurobasal so I diluted with a mixture of it and our lab's Neurobasal media (no glutamate) and plated onto 35mm dishes that had been treated with poly-ornithine for 24 hours (as explained in greater detail above).