

Cell Feeding (Observations & Info from April Horton, 9/13/04)

Neurobasal Medium (regular, *not A*) with B27, glutaMAX-1, Gentamicin and FUDR. All of her personal media has FUDR in it.

About 8-10 DIV, when glial cells are about 80% confluent, adds 2 μ l/well from 1000x stock (500 μ l/500 ml solution for Media w/FUDR). This gets the FUDR concentration up to correct level for 2 ml, then after that just feeds cells with FUDR-containing Media.

Cells are plated on Tuesdays right now.

First feeding is done by Haiwei and Irina on Monday (6 days). Cell trays are kept separate up to this point so they can feed and monitor cells.

On day 7 April starts looking at cells to judge glial coverage.

April feeds on Friday, then Monday, Friday... 3 days apart.

Adds the 2 μ l FUDR Monday or Tuesday, or maybe even Thursday on a well by well basis.

April thinks that Fibroblasts (from blood vessels) and Glial cells tend to stop dividing due to contact forces when they become confluent, so they won't necessarily overgrow the culture, but the floaty, round Microglia won't stop without the addition of FUDR.

(She also doesn't quite know what kills the neurons, whether it's a buildup of metabolites, or a loss of nutrients, but an option that Tom uses is to put in more like 3 ml of medium and feed less often.)

April throws away anything over five weeks old at this point. She puts bleach in all wells from a squirt bottle, then dumps liquid in the sink and puts the solid tray and coverslips in the biological waste bin.

Before feeding, hold plate up to the light to check for gross contamination (cloudiness in wells).

Handling trays without gloves at this point.

Look at a few wells from each plate under microscope to make sure there are cells there to feed.

Warm up enough NB medium (30-60 ml -- about 1 ml/well) in a 100 ml conical (orange-topped plastic) for about 20 minutes instead of out of stock bottle. Transfer to conical with a sterile 25 ml serological pipette. Again, don't sit pipettor upside down, and make sure not to touch tip or shaft of pipette to anything.

Glove up, spray NB medium bottle. (They use water and alcohol-proof pens for writing on the bottles.)

If being anal about contamination, used to use 2 sterile pipettes for each plate (one for removing and one for filling, I think), but it's very pipette intensive, so a bit less careful now and hasn't had a problem.

Suck out 1/2 of the media from each well with vacuum and glass pipette end. (April does this approximately right now rather than measuring out 1 ml.)

Fill 10 ml serological pipette and dispense 1 ml/well. (Often April doesn't touch the side of the well or the liquid surface when dispensing, but I noticed she does sometimes.)

April feeds from youngest to oldest since the young neurons are more valuable to her, and the first ones fed are less likely to become contaminated.

Random waste notes:

Pipettes are kept in boxes until they're ready for autoclaving and disposal since they tend to poke holes in the biological waste bags.

In neurobiology, a work-study student comes and bags up pipettes and takes the biological waste bags, then each lab has a couple days a week that they are allowed to autoclave waste, then it's taken to disposal.

Rinse used NB medium bottles, throw away caps, and put the bottles in the box by the door for recycling.

Glass waste is put in glass waste boxes (w/inner bags), then when they're full they're taped shut and put out in the hall to be carried away.