Fibril transfection in FL GFP-tau cell lines

Materials:

* Recombinant fibrils
* Opti-MEM
* Culturing medium
* HEK293 cells with GFP-0N4R tau or K18-YFP (clone 1 cells fromMarc Diamond)
* Lipofectamine 2000
* Hoescht (10 mg/ml)
* Greiner black uclear bottom 384 well plates
* Media: DMEM + 10% FBS + 0.01% Pen/Strep. Note that the Pen/Strep concentration is 1/10 of the concentration normally used in culturing.
* Doxycycline if performing in inducible cell lines (1 mg/ml in ddH2O).

Protocol

The following protocol is for 384 well format with 0N4R fibrils.

1. Check cells. Seeding works best if cells are healthy at time of splitting (60-90% confluency, split 2-4 days prior, media rosy colored)
2. Prepare lipofectamine master mix. Dilute lipofectamine for each well with opti-MEM **(0.025 uL lipo + 4.975 uL optimum=5 ul total)**. Gently mix by inverting or flicking tube. Scale up for multiple wells. Incubate for 5 min at RT.
   1. I prepare enough for 3 wells per group \* 8 dilutions plus extra. This usually works out to 0.243 ul lipofectamine + 48.357 uL opti-MEM for each group.
   2. Note: Using higher amounts of lipofectamine (>0.012ul) per well can be toxic.
3. Prepare fibril mix. Dilute **0.25 ug fibril in 5uL** final volume with opti-MEM.
4. Add lipofectamine solution to fibril solution. Pipette up and down or flick solution to mix. Incubate at RT for 20 min.
5. Fill all the wells to be used in the experiment with **60 uL** of media
6. Perform dilutions of lipo-fibril mix as desired. I usually do several 2X dilutions with opti-MEM to get seeding curves.
7. Add **10 uL** fibril/lipo mix to each well. **Change tips between each group.**
8. Cover plate and spin plates for 20 min at 1000g
9. Split cells. Resuspend in culturing media at a concentration of 3000 cells/well/10 uL.
   1. Add Doxycycline to the solution for inducible lines to give a final concentration of 10ng/ml i.e. Add to cell stock solution at **80 ng/ml**
   2. Add 10 ul of cells to each well. You don’t need to change tips between groups but try to disrupt the media already in the well as little as possible. I usually gently eject the cell solution into the well with the pipette tip touching the side of the well just above the media miniscus.
   3. Try to minimize the time between splitting the cells and completing plating to less than 1 hour. You can stagger splitting the cell lines if necessary.
10. Incubate cells for 3 days before assaying for seeding efficiency. I start to see seeding as early as 2 days but it increases over time for up to 6 days. After 3 days you run into issues with cell crowding in the well. You will need to exchange media if you go longer than 3 days.
11. Add Hoescht 1 h before reading at a final concentration of 1.1 ug/ml. Make a stock solution of 10 ug/ml in media (1:1000 dilution). Add 10 uL per well ejecting the liquid by touching the tip to the side of the well.
    1. Note: I don’t usually add Hoescht at the beginning of the assays because it interferes with cell growth/division. I sometimes add it on day3 and continue reading for several days

Well details for optimum seeding:

|  |  |  |
| --- | --- | --- |
|  | Per 384 well | |
|  | Concentration/amount | volume |
| Media |  | 60 ul |
| Lipofectamine 2000 | 0.025 ul | 10 ul |
| 0N4R fibrils | 0.25 ug |
| cells | 3000 cells | 10 ul |
|  | Total volume | 80 ul |