Lipofectamine transfection (modified from J. Wesseling´s)

Notes: Volumes are per well (12-well plates)

In my hands, it works up better up to DIV10-12 cultures (at the day of transfection).

In principle, regular media does not need to be replaced by serum-free media (as with other lipid reagents).

If toxicity is high, I leave Lipofectamine 2000 for 3-6 hours, and wash with conditioned medium that I aspirated from the same cultures before transfection (I usually supplement each well with 1ml N27, take about half the medium (around 1.5 ml) before transfection and save it in the incubator).

Protocol

1- 2-3 h before, or the day before, I supplement culture medium with 0.8-1ml warm N27.
2- Dilute **0.6**-2 µl of LF2000 Reagent into 25 µl Opti-MEM solution (*LF2000 solution*). Do not let it sit for more than **5 min**, but let it stay for at least 3 min.
3- Dilute 1 µg (range 0.5-2 µg) of DNA into 25 µl Opti-MEM (*DNA solution*).
4- Combine equal volumes of the *DNA solution* with *LF2000 solution.* I usually add DNA mix to LF mix dropwise and mix by pipetting up and down or vortexing. Let sit at RT for 20 min.
5- Add 40-50 µl per well. Before adding, remember to remove and save 1.5 ml of the conditioned medium for later.
6- Aspirate media and add 1.5 ml of the saved conditioned medium after 3-6 hours.