Reverse Transfections of Adherent Cells in 96-well Plates

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This protocol is designed as a rapid alternative to standard transfection that does not require plating cells the day before transfection. Instead, a suspension of cells is added directly to liposome complexes prepared in a 96-well plate. This protocol has been used successfully with the cells and conditions outlined below. This reverse-transfection has also been successful using larger well plates, such as 6-well plates (scaled appropriately).

This protocol is based on that from Life Technologies called: “Alternate rapid protocol for 96-well transfections without pre-plating cells”

http://tools.lifetechnologies.com/Content/SFS/ProductNotes/F_Lipofectamine%202000b-040923-RD-MKT-TL-HL050602.pdf

1. Make DNA master mix
    a. For each plasmid to be transfected, prepare a separate, sterile 1.5 mL centrifuge tube.
    b. Add Opti-MEM (Life Technologies Cat #31985070) to each tube. Use 25 µL per well to be transfected. Multiply by 1.1 to ensure enough volume is left during dispensing later.
        i. For example, if 3 wells are to be transfected with one plasmid, put 75*.1.1 uL of Opti-MEM into the tube.
        ii. We have also used regular DMEM in place of Opti-MEM.
    c. Add DNA to each tube.
        i. The original protocol calls for 320ng of DNA per well. We found that to be too much for most plasmids. Often, 50ng is sufficient, but this should be decided case-by-case.
    d. Aliquot 25 uL of the DNA master mix (es) to the 96 well plate as appropriate.
2. Make Lipofectamine master mix.
    a. As in Step *1a* above, add Opti-MEM to each tube.

b. Add 0.6 µL Lipofectamine™ 2000 (Invitrogen Cat #11668-019) per 25µL Opti-MEM (i.e. per well).
    i. For example, for transfecting 12 wells in a 96 well plate, one would add 7.2 µL lipofectamine to 300 µL OptiMEM. As above, we typically prepare 10% extra for contingency.
    ii. The ratio of lipofectamine to DNA can be optimized by altering the amount of lipofectamine added (above protocol lists 0.4 to 0.8 µL per well).
c. Incubate at room temperature for five minutes.

3. Prepare transfection complexes.
    a. Add 25 µL of the Lipofectamine™ 2000/OptiMEM mix (from step #2) to each well containing DNA (from step #1), slowly and dropwise. Mix gently by rocking the plate with hands (like a nutator).
    b. Incubate at room temperature for 30 min to allow DNA-Lipofectamine™ 2000 complexes to form.
        i. We have found that 20 minutes is often sufficient.
4. Prepare Cells.
    a. Lift cells of interest (see Subculture protocol).
    b. Resuspend to a density of 10,000 cells / 150 uL in growth medium ***without*** antibiotics.
        i. This number is typical for us in a 96-well plate for somewhat sparse confluency. This of course can be optimized for downstream assay and other contingencies.
        ii. Again, 10% extra cell suspension volume is advisable, particularly with FBS as it can cause bubbles.
    c. Gently add 150 µL of the cell suspension to each well containing the DNA-Lipofectamine™ 2000 complexes (from step #3) dropwise. Mix gently as above.
        i. You should have a total of ~200µL of liquid in each well after this step.
5. Incubate at 37^{o}C in a humidified 5% CO_2 incubator until ready to assay (24-48 h post transfection). It is not necessary to remove the complexes or change the medium. Cells will adhere as usual in the presence of the complexes.
    a. If the risk (or cost) of contamination is high, one can add PenStrep (1X penicillin/streptomycin, 10,000 I.U./mL / 10,000 µg/mL, Corning Cat #30-002-CI) ~4-6 hours post transfection. To do this, one can simply replace the non-PenStrep containing media with PenStrep (1X) containing media.
        i. This will result in the removal of the transfection reagents, but 4-6 hours should be enough time for transfection to occur successfully. This time can be extended if needed.
        ii. Most cell lines attach sufficiently to resist aspiration after 4-6 hours, but some may not.