Guidelines for Optimizing Liposome Transfection

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Some of the Focus articles in this series do not have our latest recommendations for optimizing lipid transfections. Below are considerations and recommendations for the optimization procedure.

Cell Maintenance. It is important that the cells be maintained in a consistent and healthy state prior to plating for transfection. Follow a routine subculturing procedure. We passage cultures once or twice a week at 1/10 to 1/50 dilution, depending on how rapidly the cells proliferate. Most cells should not remain at confluence >24 h.

Transfection with or without serum? For most applications, transfections are done in the absence of serum during the time of exposure of cells to the DNA-lipid complexes. Most cells are not adversely affected by a brief period (5 h) without serum. However, some transfections may have to be performed in the presence of serum. If so, prepare complexes without serum.

For LIPOFECTAMINE Reagent, transfection without serum will result in enhanced activity. Transfection in the presence of serum may change the amounts of lipid or DNA required for optimal expression. Optimize under final transfection conditions.

Typical optimization. It is best to try small scale versions of the final assay. For example, if testing different promoters for activity using CAT or β-galactosidase (β-gal) as the reporter transgene, optimize the transfection using a constitutively active vector with the same transgene reporter (*i.e.* pSV2CAT or pCMVlacZ).

For transient assays, CAT or β-gal are good reporters, with several versions of the plasmids available. For stable transfections, a *neo* expression vector with GENETICIN® selection for colony formation is standard.

Once the culture conditions are established, plate cells on 35-mm wells. Volumes given below are for 35-mm wells. If using smaller wells, change all volumes and quantities in direct proportion to surface area. Plate the cells so that they are about 80% confluent the day of transfection (which should be the next day).

The day of transfection, prepare a lipid dose response in tubes containing 100 µl serum-free medium. OPTIMEM® I is our choice for most cells, but any serum-free medium works. Even if the transfection will be done in the presence of serum, this step must be serum-free. A good dose-response for most cell types is: 1, 3, 6, 12, 24, and 36 µl of lipid reagent.

1. Prepare diluted DNA separately. The DNA should be diluted in 100 µl serum-free medium per tube (*i.e.* 0.5-2 µg per 100 µl). Even if the transfection will be done in the presence of serum, this step must be serum-free.
   The DNA can be prepared in a batch. The DNA stock is stored in water. Cesium banded, column purified, and miniprep DNA all work. We do not digest it with restriction endonucleases.

2. Pipet the diluted DNA into the diluted lipid tubes (100 µl each). Mix briefly. Incubate 15 min at room temperature.

3. Rinse the cells in medium to be used for transfection (transfection medium - either serum-free or with serum).

4. Add 0.8 ml of transfection medium to each tube containing the DNA and lipid complexes. Mix briefly. Aspirate the medium from the cells and pipet 1 ml of complexes in transfection medium onto cells. Replace in the incubator.

5. After 5 h, add 1 ml of culture medium containing twice the normal amount of serum or aspirate the lipid-DNA complexes and feed the cells fresh medium.

6. After 1-2 days expression, harvest the cultures and assay for transient expression of the transgenes or passage for stable transgene expression. For stable transfection, add selection antibiotic 72 h after the start of transfection.

Evaluating results. The transfection will result in transgene expression at various levels. We express our results on a per plate or per transfection basis rather than a specific activity basis. This allows evaluation of expression independently from survival. Determine whether or not peak activity occurred within the tested range. If not, extend testing to include the peak. If survival of cells is an issue, it is often possible to increase survival by using less lipid or DNA (or both), or by plating more cells. Each of these modifications will change the characteristics of the transfection to some extent, and further optimization can increase transfection efficiency (*i.e.* if using more cells, it might be necessary to increase the amount of lipid and/or DNA in order to achieve the level of transgene expression observed when fewer cells were transfected).

Next step. If more thorough optimization is desired, a second dose response could test a narrower range (*i.e.* 2, 4, 6, 8, and 10 µl) of lipid. The amount of DNA can be optimized as well. The number of cells plated can be changed, after which the amounts of lipid and DNA must be retested. With more cells, usually more lipid and DNA are required to obtain the same level of expression.

Cell yield. The survival of cells in the transfection should not be the first criterion for setting conditions. Usually, by adjusting plating density and reagent concentrations, conditions can be determined which are not excessively toxic to the cells. It is also possible to shorten the exposure time of the cells to the complexes, but this may decrease the activity.