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Optimize Transfections Using FuGENE® HD Transfection Reagent

Trista Schagat and Kevin Kopish, Promega Corporation

Optimal DNA delivery into mammalian cells depends on multiple factors. We recommend optimizing transfection conditions for all cell types to find the best balance between maximal protein expression and minimal impact on cell viability. This report addresses the variables that should be tested when first optimizing transfection.

A number of factors will contribute to transfection success as well as the biological response of the transfected cells. We recommend careful consideration of each of the following parameters, and illustrate successful plasmid DNA transfections using FuGENE® HD Transfection Reagent.

Cell Health: Cells should be actively dividing, passaged regularly in fresh growth medium and not allowed to become overconfluent prior to or at the time of transfection. Ideally, cells will be 75–90% confluent and greater than 95% viable (e.g., by trypan blue exclusion) at the point of harvest for transfection plating, and typically 80% confluent on the day of transfection using the FuGENE® HD Transfection Reagent. Passage number should be monitored because the cell’s biological responsiveness can be unreliable at very low or high passage numbers.

DNA Quality: Plasmid DNA used for transfections should be of high purity (A260/A280 of 1.7–1.9) with low endotoxin levels to avoid unintended cellular responses such as cytotoxicity or proinflammatory cytokine production. Preparation of plasmid DNA using a method qualified to produce transfection-grade DNA (e.g., PureYield™ Plasmid Purification Systems) will help you avoid these issues.

Transfection Method: Lipid-based reagents tend to give the lowest toxicity and have been used to transfect a wide range of cell lines. Newer reagents involve a single addition of DNA:lipid complexes to cells with no subsequent medium change. However, lipid reagents vary and the maximum protein expression and cell viability achieved can vary greatly (Fig. 1).

A final comment on optimization is to simplify transfection conditions. Choosing a reporter that is easy to assay allows for testing a range of conditions quickly with minimal variability or potential complications. Ninety-six (96)-well plates are recommended because multiple variables and replicates can be tested in a single experimental plate. Small volumes minimize the use of medium and compounds, and sensitive assays are available to detect single or multiple reporters and biological markers in a single well (1,2). Once conditions are optimized for a specific cell type, they can be scaled to other plate formats.

Optimizing Transfections with FuGENE® HD Transfection Reagent

Optimal transfection conditions for any cell line should be determined empirically. It is worthwhile to spend the time up front to ensure maximal response from the cells in all subsequent experiments. The FuGENE® HD Transfection Reagent is lipid-based, simple-to-use, and can result in high transfection efficiencies with minimal cytotoxicity. Figure 2 presents an example of optimized transfection conditions for the FuGENE® HD Reagent. Test variables include the ratio of reagent:DNA and volume of transfection mix added. The FuGENE® HD volume-to-DNA mass ratio (µl/µg) determines the charge of the mix added to the cells (the negatively charged DNA must be balanced by the positively charged, cationic lipid of the reagent), and the volume of this mixture determines how much DNA is administered. More is not necessarily better and may lead to reduced protein expression and reduced cell health (Fig. 3). Typical reagent:DNA ratios are between 1.5:1 and 4:1 with addition of 2–10µl of transfection complex per well. In this experiment, optimal conditions for HEK293 transfection were 5µl of a 2.5:1 mix.

Controls should be included in optimization experiments. Untransfected cells are used as an indication of maximum viability and no reporter expression; DNA- and reagent-only controls are included to monitor any unexpected effects of the transfection mix components on the cells.

Multiplexing to Improve Optimization

Tracking cell viability along with reporter activity is critical to determining optimal transfection conditions. High reporter activity may come at the expense of cell viability.
Fig. 2. FuGENE® HD transfection optimization experiment. Layout for a 96-well plate to determine optimal transfection conditions for FuGENE® HD Transfection Reagent. Transfection mixes (100µl) as indicated were prepared in a separate 96-well plate. Transfection mixes were transferred to the transfection plate after a 15-minute incubation (RT). All test wells contained cells in growth medium. Column 2: Control consisted of medium (10µl), DNA (10µl, 20ng/µl), or FuGENE® HD Reagent (10µl). Columns 3–11: Transfection mix with FuGENE® HD Reagent and DNA as indicated.

Fig. 3. HEK293 transfection optimization experiment. Optimal transfection conditions for HEK293 cells with FuGENE® HD Reagent were determined using the protocol outlined in Fig. 2. Cells were grown to 85% confluency, harvested, and added to a 96-well plate at 2 × 10^5 cells/100µl well. The next day, the pGL4.13 Vector (Cat.# E6681) expressing firefly luciferase was diluted in serum-free medium (DMEM, 100µl) to 20ng/µl and mixed with FuGENE® HD Reagent to achieve the indicated reagent:DNA ratio. After a 15-minute incubation, indicated volumes were added per well. The cells were mixed gently and incubated for 24 hours at 37°C/5% CO₂. Cell viability (CellTiter-Fluor™ Viability Assay) and reporter activity (ONE-Glo™ Luciferase Assay System) were assessed as in Fig. 1. Data are the average of replicate samples ± SEM.

Summary
Empirically determining optimal transfection conditions for any given cell type allows for the best return from your cell-based experiments. Optimal conditions should yield maximum reporter activity with minimum impact on cell health, thus preserving the biology of the cells for subsequent manipulation. By understanding the keys to successfully transfecting plasmid DNA, following a standard optimization plate layout, and multiplexing reporter and viability assays, ideal parameters can be determined with relative ease.

References

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<table>
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<th>Ab dilution</th>
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