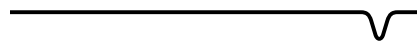


# Transfection optimization using siGLO green and siGLO red transfection indicators.



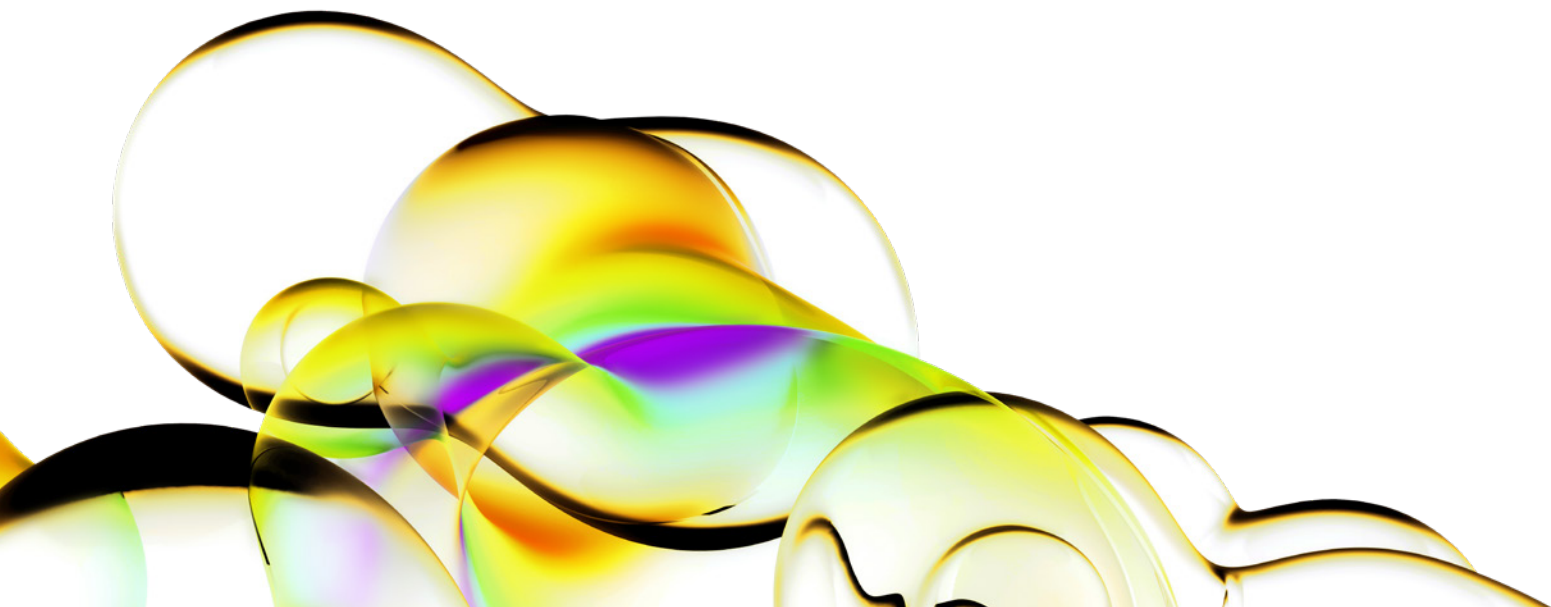
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Dharmacon siGLO Transfection Indicators (siGLO™ Green and siGLO Red) are fluorescent oligonucleotides that localize to the nucleus thus permitting unambiguous visual assessment of uptake into mammalian cells. These reagents do not engage with RISC and are not intended to provide information about siRNA function, localization or duration of silencing; however, they are ideal for defining optimal transfection conditions for siRNA or other small RNA (microRNA, crRNA).

## Introduction

Identifying optimal cell densities, lipid transfection agents, and lipid concentrations is essential for effective implementation of RNA interference (RNAi) in cell-based experiments. Poor or inefficient transfections that lead to effective gene knockdown in only a fraction of the cells can significantly hamper a researcher's ability to identify the true contribution of a gene to cellular physiology. One method of optimizing the transfection conditions for small nucleic acids employs fluorescently labeled siRNAs. These modified duplexes can be readily visualized and when combined with assays that assess the effects of transfection on cell viability, they provide users with a rapid method of identifying optimal siRNA transfection conditions. The following technical note provides a thorough description of how the siGLO Green and siGLO Red Transfection Indicators can be used to monitor overall transfection efficiencies during RNAi gene knockdown experiments.



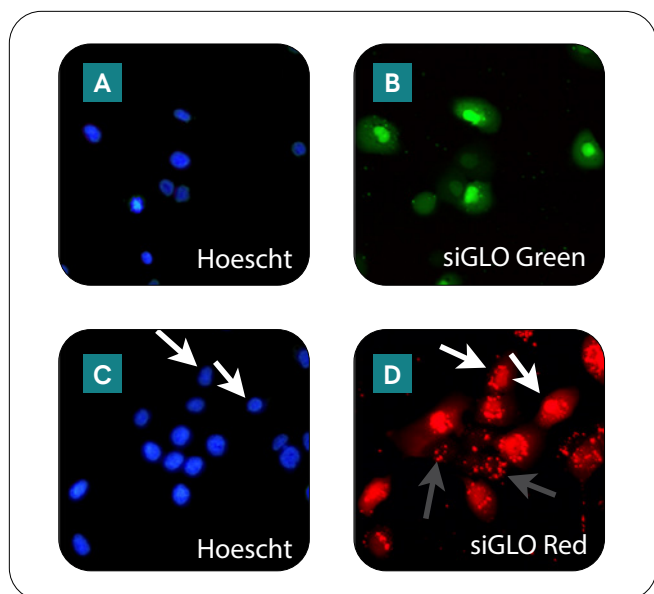


Figure 1: siGLO Green and siGLO Red show a strong nuclear staining pattern for easy monitoring of transfection. MCF 10A cells (5000/well; 96-well format) were transfected with the 50 nM siGLO Green or siGLO Red using DharmaFECT™ 1 Transfection reagent (0.15 µg/well). Cells were stained with Hoechst 33342 nuclear dye (A., C.) and images were taken 24 hours after transfection. Green nuclear staining (B.) is indicative of positive siGLO Green uptake while red nuclear staining (D., white arrows) is indicative for positive siGLO Red uptake. Additional punctate staining (D., gray arrows) can be observed in some cell types, but is not indicative of a positive transfection.

## Attributes of siGLO transfection indicators

### For a fluorescently labeled molecule to be effective

**as a transfection indicator:** 1) it must have an easy to recognize intracellular localization pattern, and 2) the most efficient conditions for transferring the fluorescent label must correlate with the best range of conditions for siRNA transfection. siGLO Green and Red Transfection Indicators exhibit both of these properties and provide a rapid, inexpensive means of optimizing cell density and lipid conditions for siRNA transfection experiments. Upon lipid-mediated transfection, siGLO transfection indicators accumulate in the cell nucleus, presenting an

easy-to-recognize staining pattern (Figure 1). Nuclear staining is widely preferred over, for instance, cytoplasmic staining, which can easily be confused with non-specific association of a fluorescently labeled molecule with the cell membrane. Equally important, optimal conditions for siGLO transfection closely parallel conditions that provide the strongest level of siRNA-mediated knockdown. These two attributes make the siGLO transfection indicators fast and inexpensive tools for optimizing conditions for gene knockdown experiments. siGLO Transfection Indicators can also be used in conjunction with a range of dyes such as nuclear Hoechst dyes (Figure 1), which stain all cells, and selective dyes like propidium iodide, which stains only dead cells.

## Selecting optimal lipid transfection concentrations and lipids using siGLO transfection indicators

Previous studies by several groups have shown that the optimal concentration of lipid during siRNA transfection can vary greatly among cell lines, and that non-optimal transfections can result in poor siRNA delivery and undesired levels of cell death. Figure 2 shows how siGLO transfection indicators can be used to identify the optimal concentration of lipid for a given transfection. Cells transfected with siGLO Green using DharmaFECT 1 were labeled with the nuclear staining dye Hoechst 33342 and then imaged to assess the success of siGLO Green uptake at each lipid concentration. The representative micrographs shown in Figure 2 allow a clear comparison of the delivery efficiency of the lipid at different concentrations. At a concentration of 0.4 µg/well, the majority of cells show a bright nuclear stain (Figure 2G), indicating transfection of siGLO Green into the cells. Concentrations of 0.2 µg/well (Figure 2F) provide slightly less efficient delivery, while 0.1 µg/well shows the poorest uptake (Figure 2E), and 0.6 µg/well (Figure 2H) shows good uptake, but also a decrease in cell viability (data not shown).

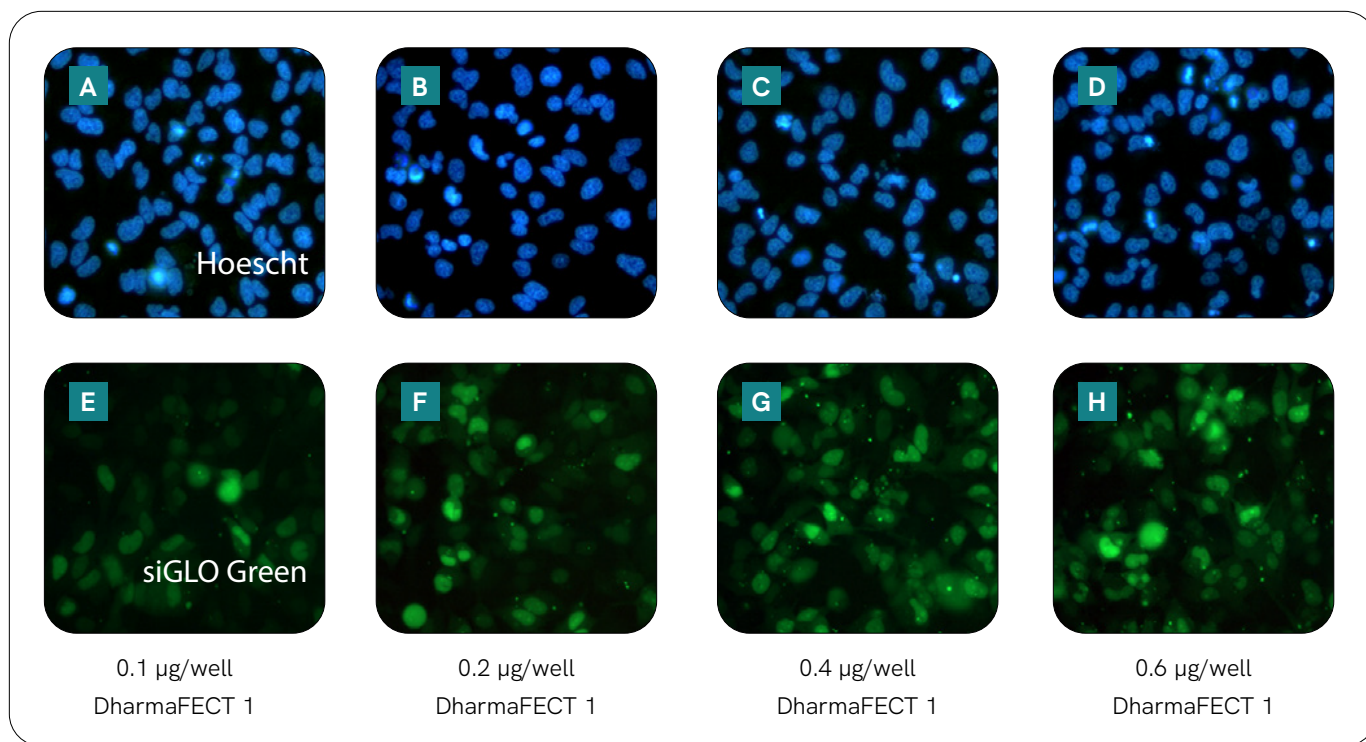


Figure 2: Lipid optimization of HeLa Cells using siGLO Green. HeLa cells were plated at 5,000 cells/well and transfected with siGLO Green overnight using DharmaFECT 1 at concentrations ranging from 0.1-0.6 µg/well. Cells were stained with the Hoescht 33342 nuclear dye and visualized by fluorescence microscopy.

siGLO Green and Red transfection indicators can also be used to identify the optimal lipid to use with your cell line of interest. As shown in Figure 3, when NIH/3T3 cells were transfected with siGLO Green using two unique cationic lipid formulations, DharmaFECT 1 and DharmaFECT 2 each lipid reagent gave a slightly different transfection profile over the range of concentrations tested (Figure 3, green bars). When these results are combined with parallel viability studies (Figure 3, blue dots) the lipid type and concentrations that provide the highest levels of transfection with minimal cytotoxicity (for example DharmaFECT 1, 0.2-0.4 µg/well) can be identified.

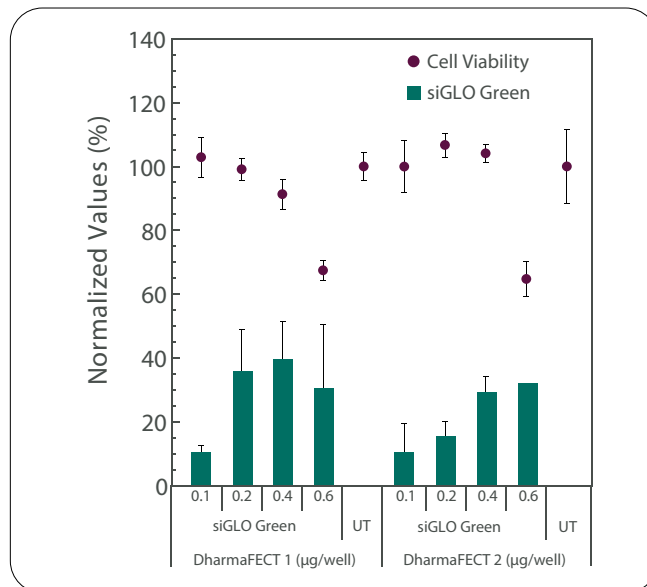


Figure 3: siGLO Green helps identify optimal lipid and concentration. NIH/3T3 cells (10,000 cells/well) were transfected with 50 nM siGLO Green using various concentrations (0.1-0.6 µg/well) of both DharmaFECT 1 and 2. The fraction of siGLO green cells was assessed after 24 hours by fluorescent microscopy and alamarBlue™ was used to assess overall cell viability. (UT = control, untreated cells).

## Correlation between optimal siGLO transfection indicator delivery and optimal gene knockdown

While optimal conditions for siGLO transfection do not guarantee gene knockdown (these are traits determined by both transfection efficiency and functionality of the targeting siRNA), conditions identified using siGLO transfection indicators repeatedly identify the very best conditions for siRNA-mediated gene knockdown. The conditions (for example DharmaFECT 1, 0.2-0.4 µg/well) that were identified to provide the best transfection of siGLO Green in NIH/3T3 cells (Figure 3) also provide the best and most efficient levels of Cyclophilin B (PPIB) knockdown with minimal cytotoxicity (Figure 4). Although the absolute percentage of siGLO-positive cells varies and is cell line-dependent, conditions that result in the highest number of siGLO-positive cells correlated with conditions that resulted in the best gene knockdown with an siRNA.

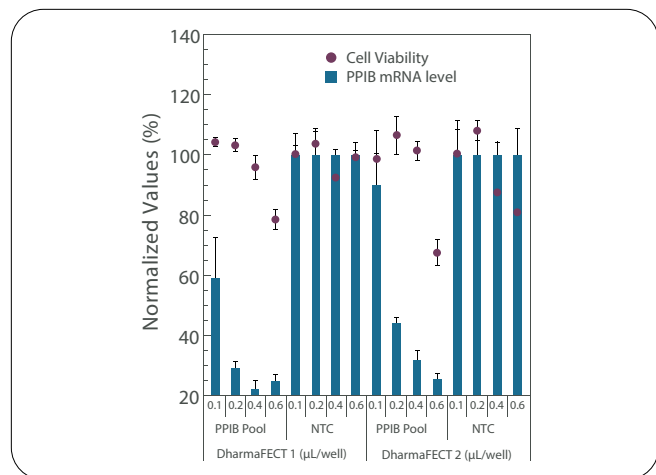


Figure 4: siGLO Green helps identify optimal lipid and concentration. NIH/3T3 cells (10,000 cells/well) were co-transfected with 50 nM siGLO Green (Figure 3) and Dharmacon™ SMARTpool™ reagent (100 nM) targeting PPIB using various concentrations (0.1-0.6 µg/well) of both DharmaFECT (DF1) 1 and 2. Overall cell viability (red circles) was assessed using an alamarBlue™ assay. PPIB expression was assessed using a branched DNA assay (Panomics). Results show that the preferred gene knockdown conditions (0.2-0.4 µg of DF1, 75-80% PPIB knockdown with less than a 5% change in cell viability) parallel conditions identified for optimal siGLO Green delivery (Figure 3).

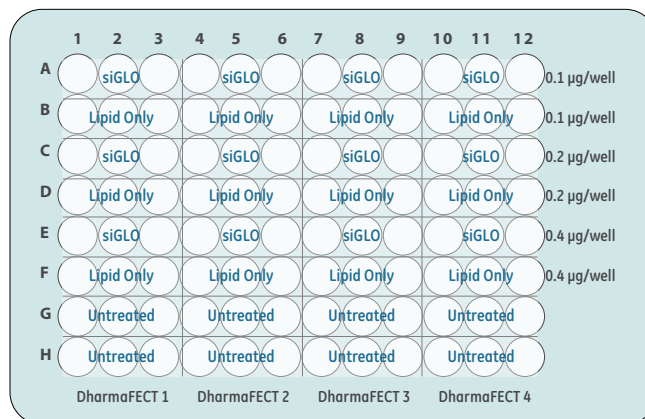


Figure 5: 96-well Plate Format to Optimize siRNA Delivery. This plate configuration allows for rapid selection of optimal transfection reagent and its concentration for siRNA transfection conditions.

## Plate formats to optimize siRNA delivery with siGLO transfection indicators

siGLO Green and siGLO Red transfection indicators can be used to optimize delivery conditions in one 96-well plate (for each cell density tested) within 24 hours. Figure 5 illustrates a 96-well layout where all of our DharmaFECT Transfection Reagent formulations can be tested simultaneously at three different concentrations in triplicate. In our lab, cell densities of 2,500, 5,000, 10,000, 20,000, and 30,000 are tested to identify the optimal concentrations for siRNA delivery.

## Summary

Identifying the most optimal conditions for lipid-mediated siRNA transfection is essential to elucidate the role(s) that individual genes play in cellular physiology. Transfection conditions that induce changes in cell viability and/or provide insufficient delivery of the targeting agent can mislead researchers toward false interpretations of data that in the long run are time consuming and costly. The siGLO Green and siGLO Red Transfection Indicators provide an inexpensive means to rapidly identify the proper lipid, lipid concentration, and cell density for your cell line of choice. When combined with readily available reagents for assessing cell viability they can accelerate the generation of meaningful data that is ready for publication.

### For more information:

If you have any questions directly related to this document, contact our experts:

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