APPLICATION NOTE

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# Determining transfection conditions using siGLO™ Transfection Indicators and the Cytell™ Cell Imaging System

Keywords: Cytell Cell Imaging System, Dharmacon, siGLO, transfection, IN Cell Investigator, high-content analysis, cell imaging

## Introduction

Dharmacon™ siGLO™ Green and Red Transfection Indicators (siGLO Green and siGLO Red) are fluorescent oligonucleotide duplexes that localize to the nucleus thus permitting simple visual assessment of transfection efficiency. They are excellent for (1) use in experiments to determine optimal siRNA transfection conditions (2) tracking individually transfected cells and (3) monitoring relative efficiency of delivery when co-transfected with siRNA. They are not, however, intended for quantitative determination of siRNA uptake.

The Cytell™ Cell Imaging System is an affordable, intuitive cell analysis instrument that streamlines a wide variety of cellular assay including cell cycle and cell viability assessments. The system provides robust quantitative results by using preconfigured software applications called BioApps. The easy-to-use, automated BioApps guide you through all steps of a cellular assay from imaging and data acquisition through to analysis, data visualization, and report generation, all at the touch of a button. Two BioApps (siGLO Green and siGLO Red) , created with MyBioApp, were used to evaluate transfection efficiency of dose-response curves for siGLO Transfection Indicators and Dharmacon™ DharmaFECT™ 1 Transfection Reagent in A549 cells.

## Materials

| Product | Cat. # |
|---|---|
| GE Healthcare Products | |
| Cytell™ Cell Imaging System | 29-0567-49 |
| siGLO™ Green Transfection Indicator | D-001630-01-XX |
| siGLO™ Red Transfection Indicator | D-001630-02-XX |
| DharmaFECT™ 1 Transfection Reagent | T-2001-XX |
| HyClone™ MEM RS medium | SH30564.01 |
| HyClone™ Ham's F12 Nutrient Mixture | SH30026.01 |
| HyClone™ L-Glutamine | SH30034.02 |
| HyClone™ Fetal Bovine Serum | SH30071.03 |
| HyClone™ Sodium Bicarbonate | SH30033.01 |
| HyClone™ DPBS w/o Ca, Mg | SH30378.02 |
| Other materials | |
| A549 Human Lung Carcinoma Cells | |
| Electron Microscopy Sciences 16% Paraformaldehyde Aqueous solution | 1570 |
| Life Technologies™ Hoechst 33342 | H3570 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| µL/well DharmaFECT 1 | 0 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0.15 | 0.15 | 0.15 | 0.2 | 0.2 | 0.2 |
| A | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| B | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| C | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| D | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| E | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| F | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| G | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| H | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| µL/well DharmaFECT 1 | 0.25 | 0.25 | 0.25 | 0.3 | 0.3 | 0.3 | 0.35 | 0.35 | 0.35 | 0.4 | 0.4 | 0.4 |

nM siGLO RNA

nM siGLO RNA

**Figure 1.** 96-well plate map of A549 cell transfection conditions. Triplicate wells containing siGLO Transfection Indicator (12.5, 25, 50, or 100 nM) and DharmaFECT 1 (0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4 µL/well) are indicated.

## Methods

### Cell growth medium

A549 cells were cultured in Complete growth medium (Ham's F-12, 2 mM L-Glutamine, 1.5 g/L Sodium Bicarbonate and 10% FBS).

### Transfection Conditions

1. A549 cells were plated in 96-well plates at 10,000 cells/well in medium containing Ham's F-12, 2 mM L-Glutamine, 1.5 g/L Sodium Bicarbonate and 10% FBS, 24 hr before transfection.
2. siGLO Transfection Indicators and DharmaFECT 1 Transfection Reagent were mixed in reduced serum medium and incubated for 20 min at room temperature.
3. Complete growth medium was added to each siGLO/DharmaFECT 1 mixture, and growth medium on the cells replaced with 100 µL of resulting transfection mix. Transfected cells were incubated for 24 hr in 37 °C 5% $CO_2$ humidified incubator, fixed with 4% paraformaldehyde, washed with DPBS, and stained with Hoechst 33342 (1 µM).

### Imaging and Analysis with Cytell

1. A 2-color BioApp was created using MyBioApp by setting the Blue Channel to image nuclei (Hoechst) and either the Orange or Green Channel to image siGLO fluorescence.
2. Nuclear reference segmentation, which overlaps the nuclear area, was chosen for the siGLO Transfection Indicators since positive signal will be localized to the nuclear area of each cell.
3. Four fields were captured from each well of the 96-well plate.
4. After imaging and initial analysis were complete, the threshold (linear gate) setting for positive and negative transfection was set immediately upstream of the strong signal observed from a histogram chart from a well containing a significant positive signal.

## Determining optimal siGLO and DharmaFECT 1 amounts for efficient transfection of A549 cells

A two-dimensional dose-response experiment was set up in a 96-well plate to determine efficient transfection conditions for A549 cells (Figure 1). A range of increasing amounts of siGLO Transfection Indicator (12.5-100 nM) and DharmaFECT 1 (0-0.4 µL/well) were used across the plate as indicated. A549 cells were plated and grown for 24 hr before the addition of the siGLO/DharmaFECT 1 mixture. After an additional 24 hr cells were fixed, stained and analysed on the Cytell Cell Imaging System.

## Imaging and Analysis with Cytell Cell Imaging System

Using MyBioApp a simple 2-color BioApp was developed for each siGLO Transfection Indicator and named siGLO Green and siGLO Red, respectively. Since siGLO Transfection Indicators become localized to the nucleus in positive transfections, nuclear reference segmentation was applied as a mask to sample the siGLO fluorescence signal. Example images of negative and positive transfections are shown in Figure 2.

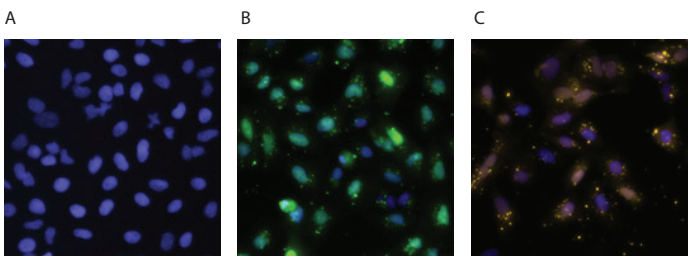
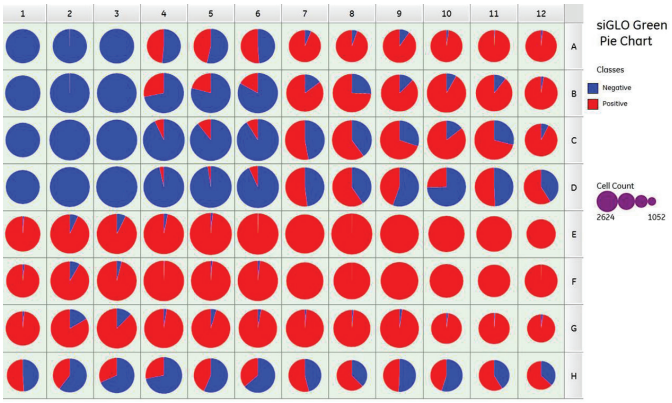
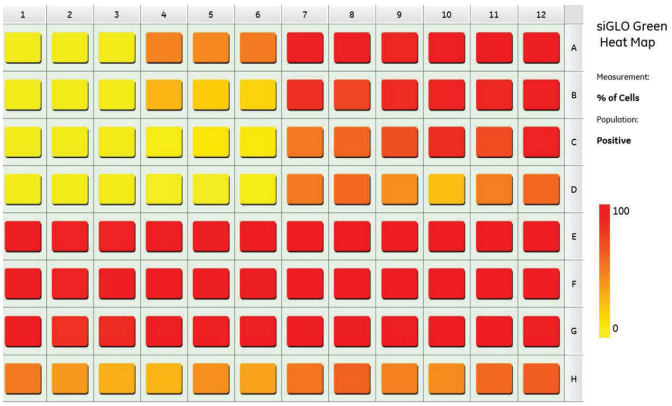



**Figure 2.** Images of negative and positive transfection of A549 cells. **A.** Negative Controls, in which no DharmaFECT 1 and up to 100 nM siGLO Transfection Indicator was added, show no nuclear siGLO fluorescence for either siGLO Green or siGLO Red Transfection Indicator; representative image shown. **B.** Positive transfection staining in the nuclear region for Hoechst (blue) and siGLO Green Transfection Indicator (green). **C.** Positive transfection staining in the nuclear region for both Hoechst (blue) and siGLO Red Transfection Indicator (orange).

A




B




C

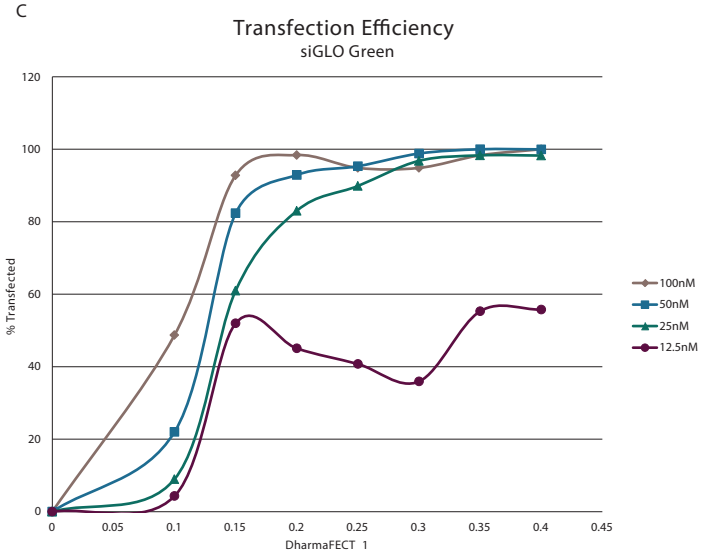
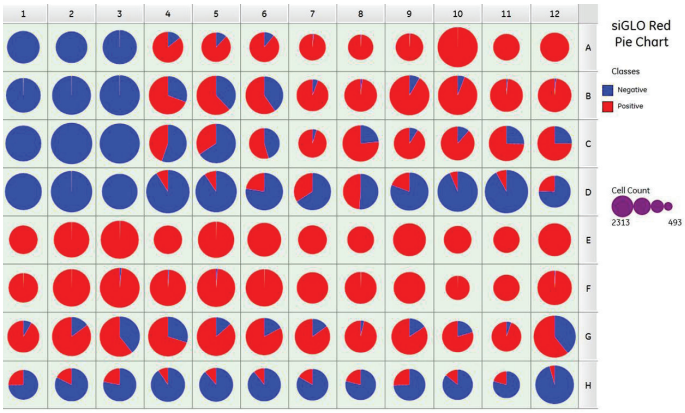



**Figure 3.** Transfection results for siGLO Green. **A.** Pie chart view showing negative (blue) and positive (red) transfection results. In addition, the BioApp has been set up to automatically size each pie diagram to indicate number of cells counted. **B.** Heat map showing percentage of siGLO Green Transfection Indicator positive cells. **C.** A graph of transfection efficiency versus DharmaFECT 1 concentration for 4 concentrations of siGLO Green Transfection Indicator.

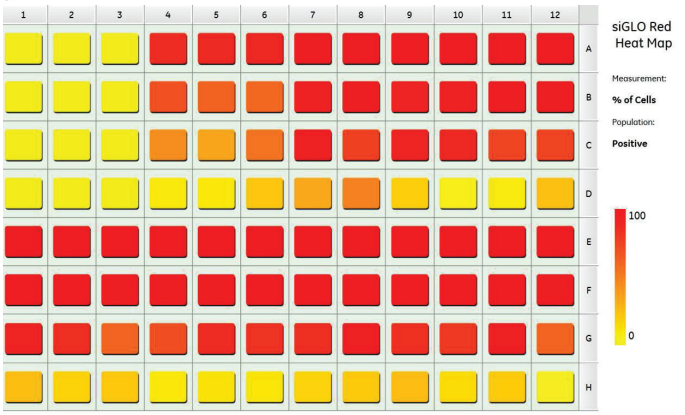
## siGLO Green results

The pie chart view on Cytell Cell Imaging System allows an easy and informative means of evaluating the siGLO Green transfection results (Figure 3A). It can clearly be seen that both increasing amounts of siGLO Transfection Indicator and DharmaFECT 1 produce higher percentages of transfected A549 cells. When no DharmaFECT 1 is added, no transfection of siGLO Transfection Indicator is observed (wells A-D; columns 1-3). As more DharmaFECT 1 is added, siGLO transfection is increased in all levels of siGLO Green. An average of triplicate wells shows transfection efficiency reaching greater than 90% for 25 nM and above with 0.25 µL of DharmaFECT 1 (Figure 3C). For varying amounts of siGLO Green Transfection Indicator, average percent transfection is plotted as a function of DharmaFECT 1 concentration in Figure 3C.

A




B




C

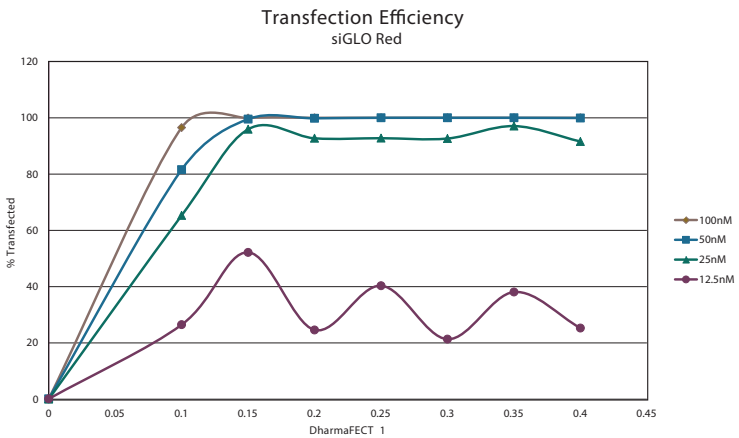



**Figure 4.** Transfection results for siGLO Red. **A.** Pie chart view showing negative (blue) and positive (red) transfection results. In addition, the BioApp has been set up to automatically size each pie diagram to indicate number of cells counted. **B.** Heat map showing percentage of siGLO Red Transfection Indicator positive cells. **C.** A graph of transfection efficiency versus DharmaFECT 1 concentration for 4 concentrations of siGLO Red Transfection Indicator.

## siGLO Red results

As expected, the siGLO Red Transfection Indicator results (Figure 4) are very similar to those observed for siGLO Green (Figure 3). Again, increased transfection is seen by increasing both siGLO Transfection Indicator and DharmaFECT 1. Pie chart size offers an immediate visual indication of determining whether either reagent had an effect on cell growth. Neither high amounts of siGLO Transfection Indicator nor DharmaFECT 1 had significant effect on cell growth under these conditions. The heat maps for both siGLO Transfection Indicators allow an easy way to select favourable conditions for evaluating transfection of siRNAs. As can be seen by the large number of red colored wells, there is a broad range of favourable conditions. The average percent of transfection of varying amounts of siGLO Red with increasing DharmaFECT 1 amounts from triplicate wells is shown in Figure 4C. As expected, both siGLO Transfection Indicators performed similarly with respect to siGLO and DharmaFECT 1 amount added. This allows a choice when pairing with siRNAs and performing fluorescent phenotypic assays used to evaluate knockdown effects.

## Summary

The Cytell Cell Imaging System provides a rapid and easy means of evaluating transfection efficiency using Dharmacon siGLO Transfection Indicators. A quick scan of the Pie Chart or Heat Map allows the user to make decisions about the amount of transfection reagent to use to begin evaluating unlabelled siRNAs targeted to genes of interest. The user can also have confidence that the transfection conditions did not have an adverse effect on cell growth. The Cytell Cell Imaging System and Dharmacon siGLO Transfection Indicators provide a rapid, affordable, and easy means to analyse transfection efficiency when setting up a siRNA experiment.


**If you have any questions, contact**

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