

## Multimode Detection

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## Determination of Transfection Efficiency using the EnSight Multimode Plate Reader

### Introduction

Cell-based assays often rely on the (co-) expression of certain receptors introduced in a transfection process. Also the

generation of cells for reagent production requires well controlled expression conditions. Therefore the optimization of transfection rates and monitoring of cytotoxicity effects are highly important for the analysis of gene-of-interest expression levels and reproducibility

The imaging capabilities of the EnSight™ multimode plate reader are ideally suited to the optimization of protocols for transfecting plasmids into eukaryotic cells. The combination of brightfield and fluorescence imaging modes allows fast and direct quantification of cell viability and transfection efficiency (using a fluorescent protein marker) in a non-invasive, automated format. It avoids the use of a nucleic acid stain, which can result in additional cell stress, therefore enabling the same samples to be used in multiple downstream applications.

Optimization of transfection conditions can be a time-consuming part of protocol development. The EnSight system's fast multiplexing capability and ability to monitor cells at multiple time points under multiple conditions of optimization can reduce the time required for this critical process from a week to 1-2 days.

In this application note, we demonstrate the use of the EnSight system for optimization of transient transfection protocols for human embryonic kidney (HEK293T) cells.

## Materials and Methods

Figure 1 shows the assay protocol used to optimize the transfection conditions using EnSight imaging technology. The images are analyzed automatically during measurement using a custom-designed method "Brightfield TransfectionAnalysis" in Kaleido, the instrument software.

The image acquisition and analysis process is shown in Figure 2. Brightfield and green fluorescence (GFP)

images were acquired in channel 1 and 2 respectively. Thresholds for all analyses were selected according to the negative control (non-transfected cells). Transfection efficiency and cell viability were calculated.

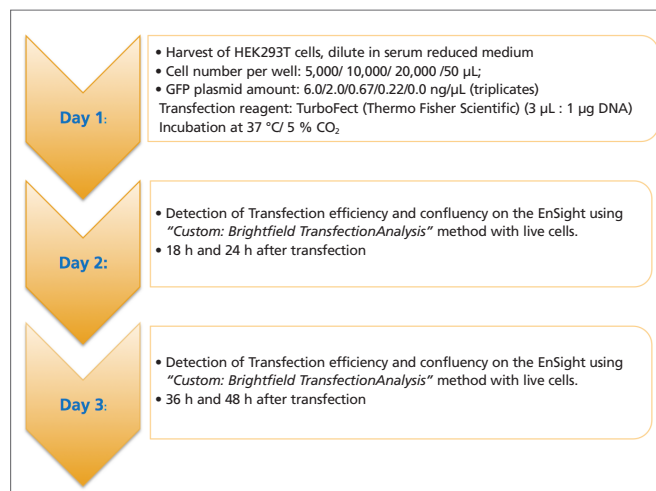


Figure 1. Transfection protocol for HEK293T cells transfected with a GFP plasmid.

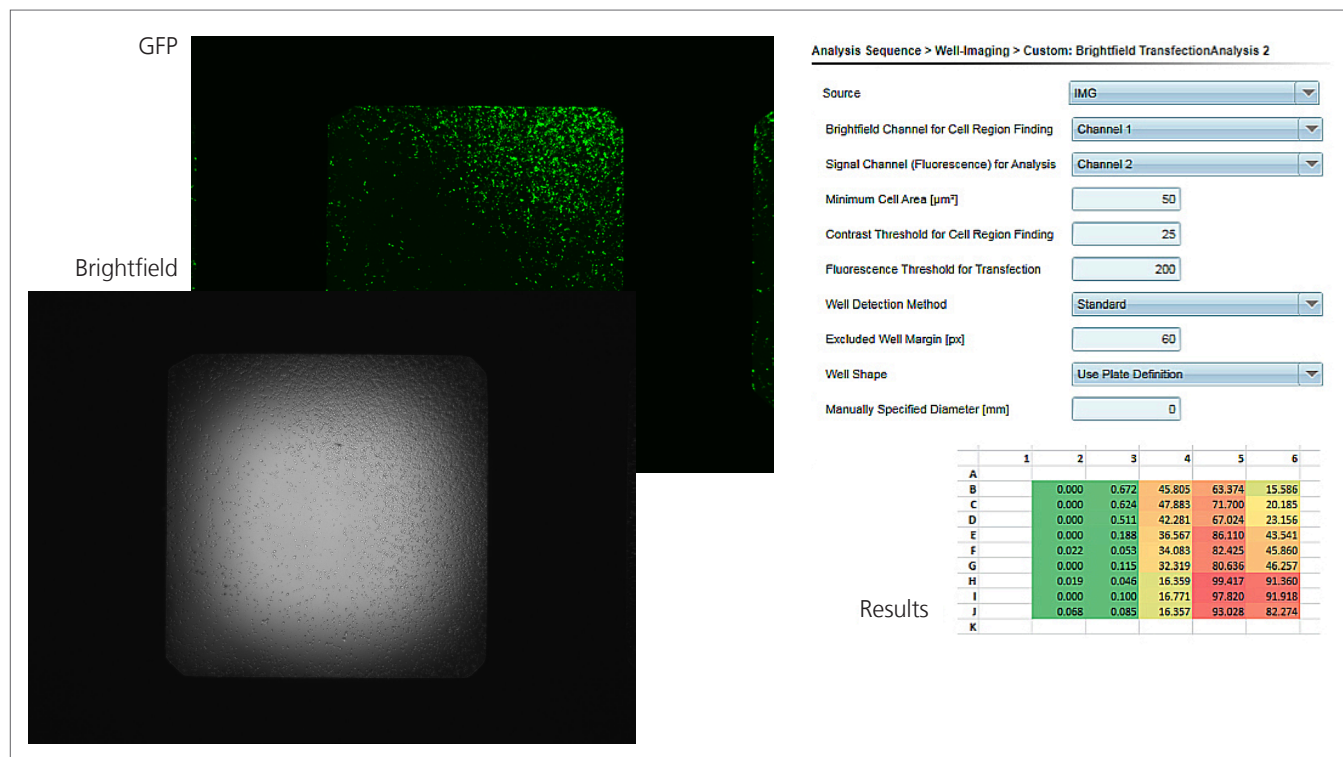


Figure 2. Image acquisition and analysis method. The image analysis first localizes the area of the well covered by cells (confluency determination) using brightfield imaging and secondly in the cell-covered area, finds the region that shows a fluorescence signal above a user-defined threshold. The size of this region compared to the size of the cell covered region is reported as transfection.



## Results

Overlays of a representative GFP fluorescence and brightfield image are shown in Figure 3 and, for different time points, in Figure 4.

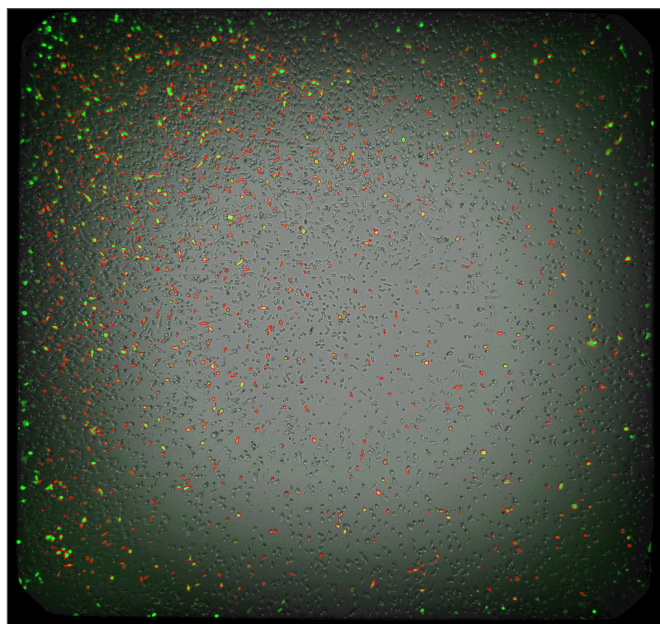


Figure 3. HEK293T (10,000 cells/well) in 384-well plates. 18 hours post transfection. Brightfield imaging (grey) is used to localize cell covered regions; GFP-fluorescence (green) to find the 'transfected' part of the cell covered regions. Confluency of cells is about 68%; 5.3% of this area shows emission of fluorescence indicating a 5.3% transfection rate. Display of the analysis used contrast setting features of Kaleido software which are in development at the time of publication. Field of view has been cut to approx. the size of the well area.

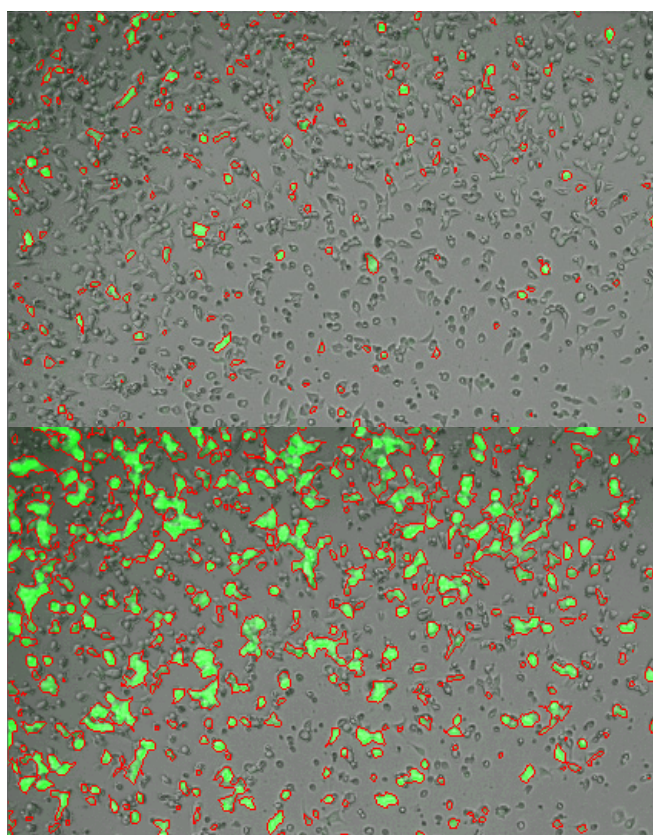


Figure 4. Detailed views of the same well as in Figure 1. 18 hours (top) and 36 hours (bottom) post infection. The degree of confluency decreased slightly from 68% to 65%. Visible transfection rate increased from 5.3% to 24.6%, while the brightness of the fluorescent regions increased almost two-fold.

The calculated transfection efficiency and confluency of HEK293T cells at 24 hours post transfection are shown in Figure 5 for a range of starting cell densities.

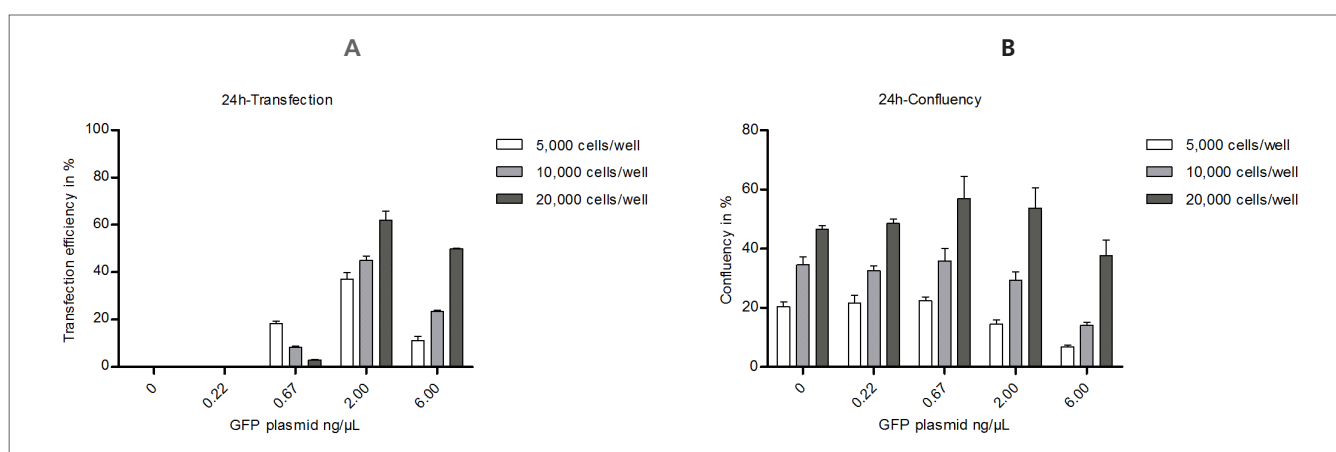


Figure 5. Calculated transfection efficiency (A) and confluency (B) at 24 hours post transfection, (N=3).

At 24 hours, optimal transfection results were observed using 2 ng/μL GFP plasmid DNA at all seeding densities. Incubation with 6 ng/μL GFP plasmid DNA resulted in reduced transfection efficiency compared to 2 ng/μL GFP plasmid DNA and was also associated with a reduction in confluency. In general, lower cell numbers showed higher sensitivity to potential cytotoxic effects caused by increasing GFP plasmid DNA concentration, but with low concentration transfection rates were higher. At 24 hours, cells seeded at 20,000 cells showed the highest transfection efficiency and confluency.

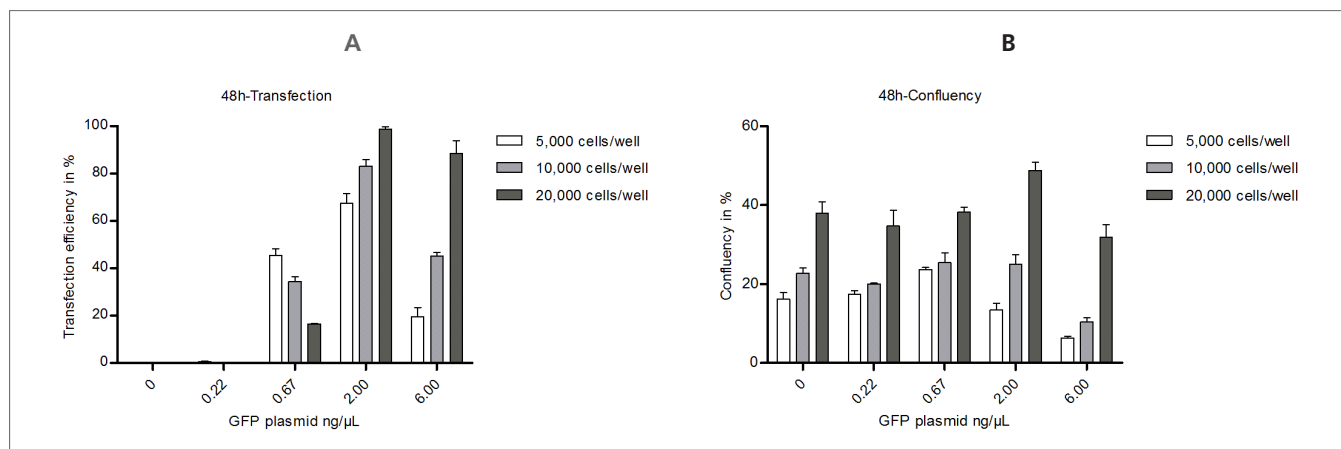


Figure 6. Calculated transfection efficiency (A) and confluency (B) at 48 hours post transfection, (N=3).

Minimal changes in overall confluency were observed between 24 and 48 hours under non-toxic conditions (0 to 2 ng/μL GFP plasmid DNA), consistent with the use of serum reduced medium (Figure 5A and 6A). At 48 hours, the optimal transfection conditions were at 2 ng/μL GFP plasmid DNA, confirming results of the 24 hour time-point, and the efficiency approached 100% at 20,000 cells/well. A substantial toxic effect at 6 ng/μL GFP plasmid DNA was observed for 5,000 and 10,000 cells/well.

## Conclusion

This study demonstrates how the EnSight multimode plate reader can be used as part of a cellular reagent generation program to identify the optimal conditions for plasmid transfection into a commonly used cell line (HEK293T cells). Transient transfection is a widely used method for (over) expression of genes of interest and, combined with the use of a characterized cell line, can allow the development of a stable and robust assay that can also be used for the purpose of high throughput screening using other measurement technologies such as Alpha, LANCE® or luminescence.

Conditions optimized to ensure low cytotoxicity reduce the risk of unwanted side-effects, which can occur as a result of cytotoxic

stress. Optimized conditions improve the reproducibility and increase the level of expression of the gene of interest in transfected cells, therefore allowing more uniformity, for example, during testing of compounds in a high throughput screen. Tracking of transfection efficiency gives an additional control for compound efficiency data, as it directly impacts signal intensity.

The key advantages of using multiplexed imaging of orthogonal fluorescence and brightfield channels in facilitating the reagent generation workflow were:

1. Cell viability and transfection efficiency could be quickly and accurately assessed in parallel
2. Multiple time points can be analyzed within one experiment using a single preparation of cells in a non-invasive way
3. Cytotoxic effects resulting from the transfection reagents could be characterized
4. Total time to optimize conditions for the cell line was only 48 hours, compared to a week when run using conventional endpoint measurements of cell viability