GFP transfection efficiency assay using 293T and Propidium Iodide

1. Introduction

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. In modified forms it has been used to make biosensors, and many animals have been created that express GFP as a proof-of-concept that a gene can be expressed throughout a given organism, or in selected organs or cells or interest. GFP can be introduced into animals or other species through transgenic techniques, and maintained in their genome and that of their offspring.

The Countstar® Rigel system is a smart, intuitive cell analysis instrument that streamlines a wide variety of cellular assay including transfection, apoptosis, cell surface marker, cell viability and cell cycle assessments. The system provides robust fluorescence quantitative results and high quality image. The easy-to-use, automated procedure guide you to complete a cellular assay form imaging and data acquisition through to analysis, data visualization, and report generation, all at the touch of a button. GFP transfection application was used to evaluate transfection efficiency of cells.

2. Material

Countstar® Rigel,
Countstar®slider
293T human renal epithelial cell line
pcDNA3.1-GFP plasmid Transfection reagent

3. Method

3.1 Transfection Conditions

(1). 293T cells were plate in 12-well plates at 200,000 cells/well in medium containing DMEM, 2mM L-Glutamine, 1.5g/L sodium Bicarbonate and 10% FBS incubated for 24 hours in 37℃ 5% CO2 humidified incubator before transfection.

(2). Plasmid and transfection reagent were mixed in reduced serum medium and incubated for 20 min at room temperature. A two-factor dose-response experiment was set up in a 12-well plate to determine efficient transfection conditions for 293T cells (Figure 1). A range of increasing amounts of transfection reagent (0-2 μl) and plasmid (0.5-2 μg) were used across the plate as indicated.

(3). Add the mixture into plate. Transfected cells were incubated for 24 hours in 37℃ 5% CO2 humidified incubator.

(4). Harvested the cell after incubated.

µl/well transfection reagent	1	2	3	4
	0	1	1.5	2
A	0.5	0.5	0.5	0.5
B	1	1	1	1
C	2	2	2	2

µg Plasmid

Figure 1 12-well plate map of 293T cell transfection conditions. Signal well containing transfection reagent (0, 1, 1.5, or 2 µl) and plasmid (0.5, 1, or 2 µg) are indicated.

3.2 Imaging and analysis with Countstar® Rigel

(1) A signal-color application procedure was created by setting Green channel to image GFP fluorescence.

(2) 3 fields were captured from each chamber.

(3) After imaging and initial analysis were complete, the threshold (log gate) setting for positive and negative transfection was set by FCS software.

4 Result

4.1 Images captured by Countstar® Rigel imaging system

A signal-color application procedure was created by setting Green channel to image GFP fluorescence, plus a bright field. Bright field picture reference segmentation was applied as a mask to sample the GFP fluorescence signal. Example images of negative and positive transfections are shown in Figure 2.

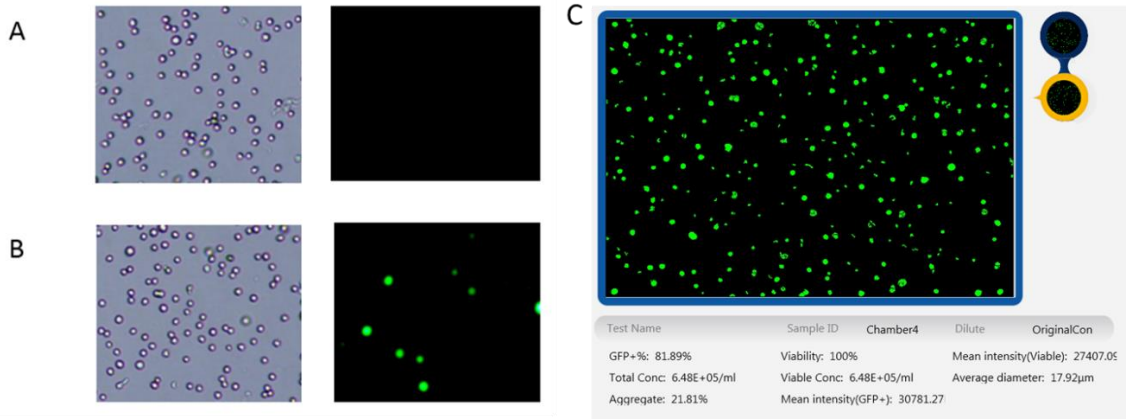


Figure 2 Images of negative and positive transfection of 293T cells. A. Negative controls, in which no transfection reagent and up to 2 µg plasmid, show no GFP fluorescence signal; representative image shown. B. Positive transfection staining for GFP fluorescence; C Screenshot of result on Countstar Rigel

4.2 Efficiency of GFP transfection

The pie chart view allows an easy and informative means of evaluating the transfection results (Figure 3A). It can clearly be seen that both increasing amounts of transfection reagent and plasmid produce higher percentages of transfected 293T cells. When no transfection reagent is added, no

transfection of GFP signal is observed (wells A-C; column 1). As more plasmid is added, transfection is increased in all levels of green signal. For varying amounts of transfection reagent, percent of transfection is plotted as a function of plasmid concentration in Figure 3B.

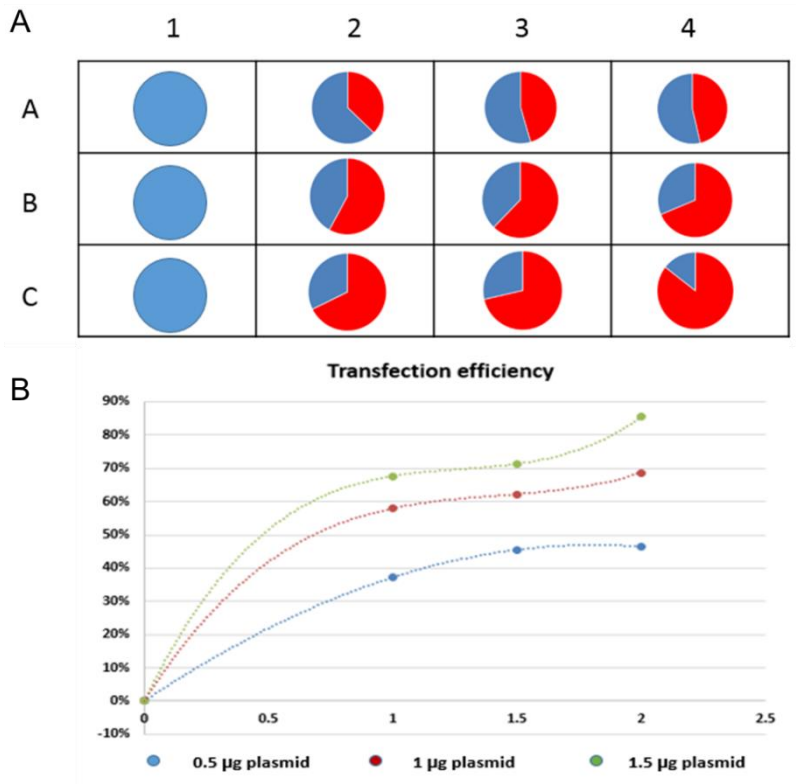
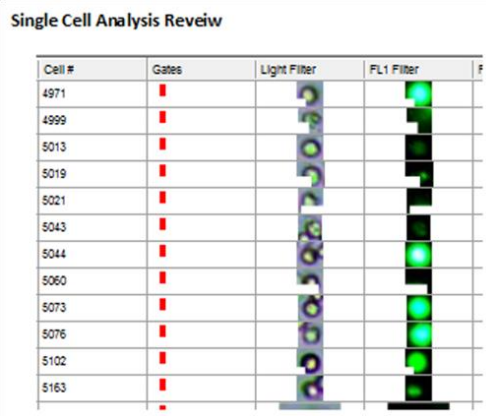


Figure 3 Efficiency of Transfection results. A. Pie chart view showing negative (blue) and positive (red) transfection results. D. A graph of transfection efficiency versus transfection reagent concentration for 3 concentrations of plasmid.

4.3 Analyzed fluorescence intensity by FCS express

Use FCS express to analysis the data like flow cytometry, gating the cells dependent on the cell population and fluorescence intensity (Figure 4A), the FCS express also supplies the function to review every signal cell, validation the data through the image (Figure 4B).

A



B

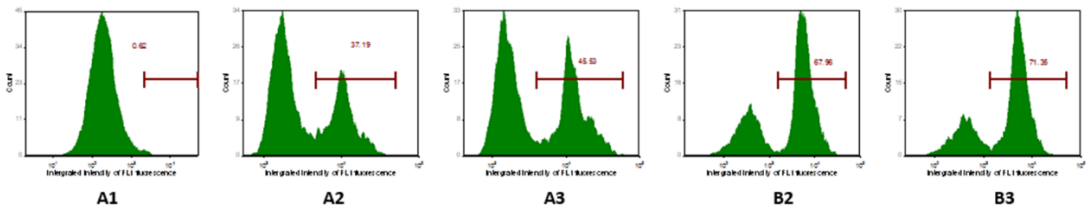


Figure 4 Review the result in FCS Express. (B). Review every signal cell and validate the data through the image. (C) FCS express analysis results of transfection, gating the cells dependent on the cell population and fluorescence intensity.

5. Conclusion

The Countstar Rigel system provides a rapid and easy means of evaluating transfection efficiency. FCS express supplies the function to review every signal cell, validation the data through the image. The user can also have confidence to carry out next experiments based on Countstar Rigel results. Countstar Rigel also can be used for analyzing transfection efficiency when setting up a siRNA experiment.