LUNA-FX7™ Automated Cell Counter, Transfection Efficiency, HEK293 cells, GFP/RFP Transfection, Dual-color Fluorescence, Image Cytometry, Interactive Thresholding, Fluorescence Gating, Linearity, Negative Control, Double-positive Cells, Single-color Transfection, Benchtop Cell Analysis

Rapid and Reliable Evaluation of Transfection Efficiency Using the LUNA-FX7™ Automated Cell Counter

INTRODUCTION

Transfection — the delivery of foreign genetic material into eukaryotic cells — is a fundamental technique in modern molecular biology, underpinning applications ranging from protein expression studies to gene therapy development. However, because transient transfection efficiency is inherently variable, quantifying the proportion of successfully transfected cells is essential for ensuring reproducibility and comparability across experiments.

Fluorescent reporter genes such as GFP and RFP are widely used as rapid, reliable indicators of successful gene delivery. Traditionally, flow cytometry has been the gold standard for quantifying transfection efficiency, but its high cost, large sample volume requirements, and steep learning curve present barriers for many laboratories. The LUNA-FX7™ Automated Cell Counter addresses these limitations by combining high-resolution image cytometry with dual-channel fluorescence detection, offering a streamlined benchtop alternative.

In this application note, we demonstrate the accuracy and flexibility of the LUNA-FX7™ for evaluating single-color (GFP or RFP) and dual-color (GFP+RFP) transfection efficiency using HEK293 cells. Quantitative performance was validated by directly comparing LUNA-FX7™ measurements against flow cytometry across all cell populations.

MATERIALS

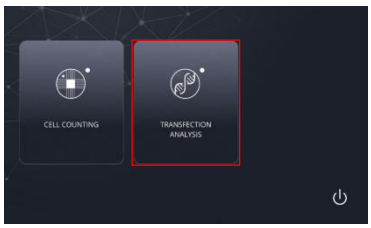
Item	Details
Cell line	HEK293 (Human Embryonic Kidney 293)
Transfection reagent	Lipofectamine™ 3000 Transfection Reagent (L3000001)
Plasmids	GFP-expressing plasmid; RFP-expressing plasmid
Slides	LUNA™ 1-Channel Slides
Instrument	LUNA-FX7™ Automated Cell Counter
Culture medium	DMEM supplemented with 10 % FBS

Experimental Procedures

1. Maintain HEK293 cells in optimal condition following the manufacturer's recommended transfection protocol.
2. Perform single-color (GFP or RFP) or dual-color (GFP+RFP) transfection alongside non-transfected negative controls.
3. Harvest cells 48 hours post-transfection by trypsinization and resuspend in an appropriate buffer.
4. Load a transfected sample onto a LUNA™ 1-Channel Slide to determine the optimal fluorescence exposure level, and save these settings to the protocol.
5. Load the negative control cells. Using the protocol established in Step 4, press "Set Negative Control" to establish the baseline fluorescence.
6. Load transfected samples onto a new slide at the same exposure settings and press "Count Sample" to begin analysis.

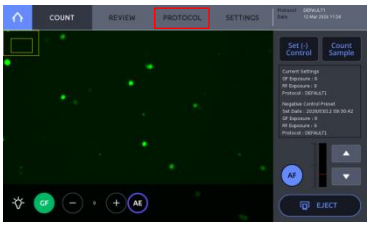
Note. For linearity validation, pure GF+, RF+, and GF+RF+ (double-positive) cell populations were each mixed with non-transfected cells at theoretical ratios of 0 %, 25 %, 50 %, 75 %, and 100 %.

1

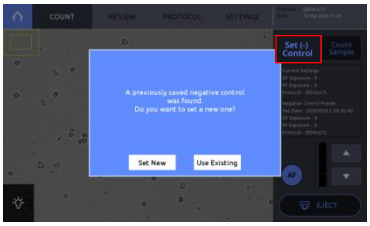


From the main instrument menu, select the **TRANSFECTION ANALYSIS** mode.

2

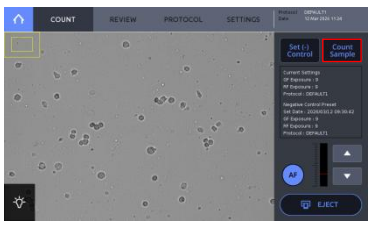


Load a transfected sample, optimize the exposure level, and save these settings under the Protocol menu.



Ensure the protocol from Step 2 is selected, then load the negative control and press "Set (-) Control."

4



Load the transfected sample onto a new slide. Using the established protocol, press "Count Sample" to begin the analysis.

5

Review the histogram distributions on the results screen and use the color-coded images to visually validate the tagged cell populations.

RESULTS

Precise Identification of Fluorescent Populations via Interactive Gating

The LUNA-FX7™ demonstrated high sensitivity in detecting cells across a broad range of fluorescence signal intensities, successfully distinguishing transfected cells from background even when expression levels were low. Using the interactive histogram interface, users can directly control fluorescence detection boundaries to suit their experimental requirements.

Setting the threshold to a lower position captures both strongly and weakly expressing cell populations, ensuring maximum detection sensitivity. Conversely, raising the threshold applies a more stringent cutoff, restricting detection to cells with high fluorescence intensity and effectively excluding dim or weakly positive populations. The color-coded brightfield overlay images confirm that this histogram-based gating approach enables precise and user-defined isolation of target cell populations (Figure 1A and 1B). This flexibility is particularly valuable when comparing experiments with varying expression levels or when strict positive cell criteria are required.

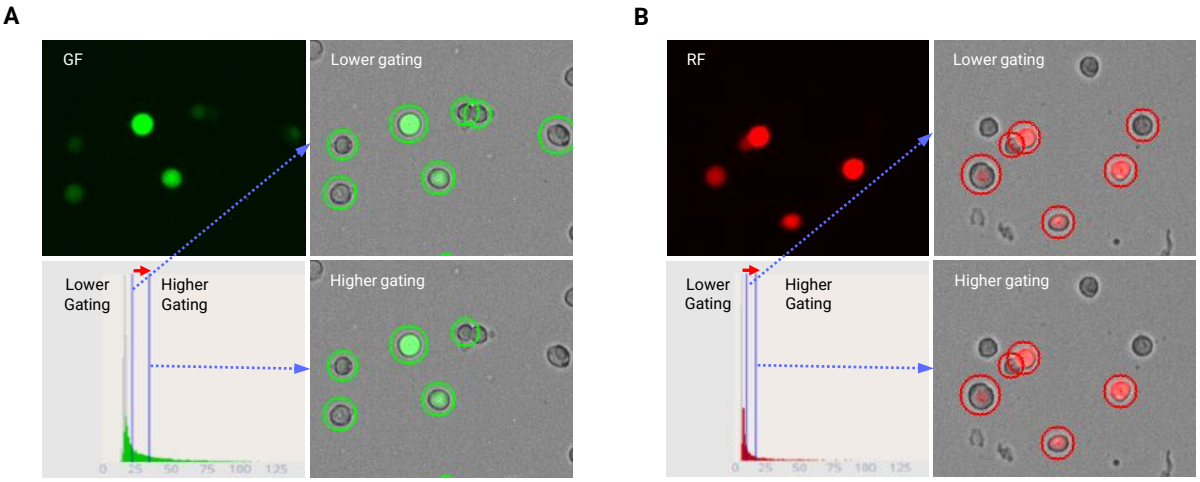


Figure 1. *Representative fluorescence images of GF (A) and RF (B) and corresponding histograms illustrating Lower and Higher Gating thresholds. Dashed lines indicate the gate-to-image correspondence. Green and red circles denote detected GF+ and RF+ cells, respectively. Yellow triangles (▲) indicate cells detected exclusively under lower gating.*

Strong Correlation Between LUNA-FX7™ and Flow Cytometry Measurements

To validate the quantitative accuracy of the LUNA-FX7™, the same GF+, RF+, and GF+RF+ cell mixtures were analyzed in parallel using flow cytometry. LUNA-FX7™ measurements were plotted against flow cytometry values across all mixing ratios, and linear regression analysis revealed near-perfect correlations for all three cell populations: $R^2 = 0.9856$ for GF+ cells (Figure 2A), $R^2 = 0.9913$ for RF+ cells (Figure 2B), and $R^2 = 0.9967$ for GF+RF+ double-positive cells (Figure 2C). These results demonstrate that the LUNA-FX7™ produces quantitatively equivalent measurements to flow cytometry across single- and dual-color fluorescence channels, confirming its reliability as a benchtop alternative for transfection efficiency analysis.

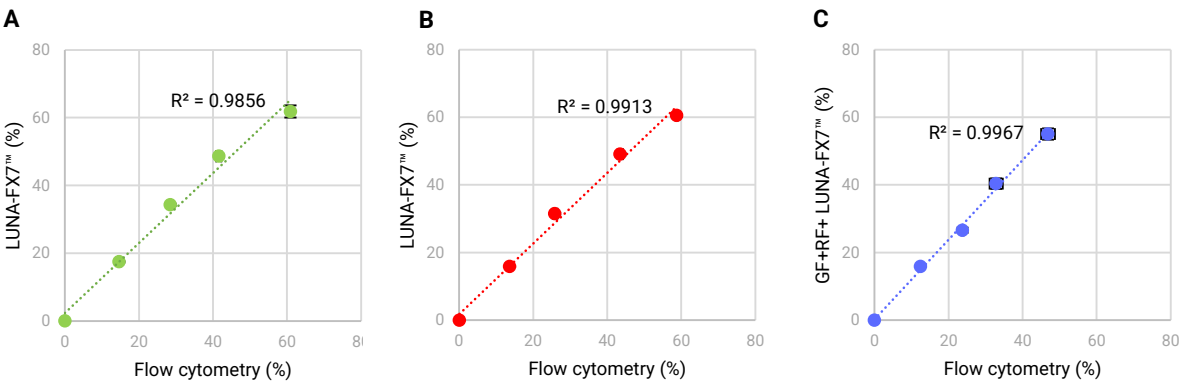


Figure 2. *LUNA-FX7™-measured cell percentages were plotted against flow cytometry values for (A) GF+ ($R^2 = 0.9856$), (B) RF+ ($R^2 = 0.9913$), and (C) GF+RF+ double-positive cells ($R^2 = 0.9967$). Data points represent the mean ± SD of three independent replicates. Dotted lines represent linear regression fits.*

CONCLUSION

The LUNA-FX7™ Automated Cell Counter offers a robust, user-friendly benchtop solution for accurate and flexible transfection efficiency evaluation. This application note demonstrates that the instrument reliably quantifies GF+, RF+, and GF+RF+ double-positive HEK293 cell populations with exceptional linearity ($R^2 \geq 0.98$) across the full 0–100% expression spectrum. The interactive gating feature provides additional flexibility, allowing researchers to tailor detection thresholds to their specific experimental criteria — whether prioritizing sensitivity or stringency.

By analyzing 47 high-resolution images per sample, the LUNA-FX7™ ensures statistically robust results even for heterogeneous samples or experiments with low transfection rates, making it a practical and accessible benchtop alternative to flow cytometry for single- and dual-color transfection analysis.