Monitoring protein expression and transfection efficiency using flow cytometry

Molecular biology applications on the BD Accuri™ C6 Plus flow cytometer

Features

- Monitor gene and protein expression easily using flow cytometry
- Generate quantitative data to complement results from immunoblotting and cell imaging experiments

Flow cytometry is a powerful and invaluable tool for cell biologists studying gene expression and regulation. With its ability to assess protein expression at the single-cell level, flow cytometry can be employed to obtain a quantitative measure of gene expression in experimental systems. This data can complement and extend the findings of more conventional methodologies such as immunoblotting and cell imaging in assessing gene modulation. On the BD Accuri™ C6 Plus personal flow cytometer, you can perform these assays right on your benchtop. Importantly, flow cytometric protocols are often easier and faster than conventional cell analysis methodologies.

Flow cytometry also provides the ability to measure the percentage of cells in culture in which the gene of interest has been successfully modified. For example, using CRISPR-Cas technology to edit a gene tagged with a fluorescent reporter, you can determine the percentage of cells in which the gene has been successfully knocked out by assessing the reduction in reporter expression.

The BD Accuri C6 Plus can also assess reduction in protein expression obtained via transcript (RNA) silencing methodologies. Figure 1 shows an experiment in which human embryonic kidney cells were reverse transfected with an siRNA targeting cyclin B1. Cells were collected after 24 and 48 hours and split for analysis by Western blot or flow cytometry. The immunoblotting results (Figure 1A) show near complete loss of cyclin B1 expression after 24 hours, and complete loss of expression after 48 hours, in contrast with cells treated with a scrambled siRNA reagent, or untreated cells.

Results from the BD Accuri C6 Plus (Figure 1B) showed that the mean fluorescence intensity of cyclin B1$^+$ cells, correlating with the protein's level of expression, decreased from 24 to 48 hours, consistent with the immunoblotting data. In addition, flow cytometry-based analysis allowed the quantitation of cells in culture that expressed cyclin B1 post-treatment (36.4% and 7.7% after 24 and 48 hours, respectively), and thus for a more quantitative estimate of the reduction in cyclin B1 expression.

Efficient uptake of plasmids or vectors by cells in culture is critical for successful downstream molecular biology applications. Conventionally, researchers optimize transfection in an empirical process in which different amounts of transfection reagent, foreign DNA and varying ratios between the two need to be tested. Consequently, optimization of transfection conditions can become a tedious and lengthy process that consumes precious (and expensive) reagents.

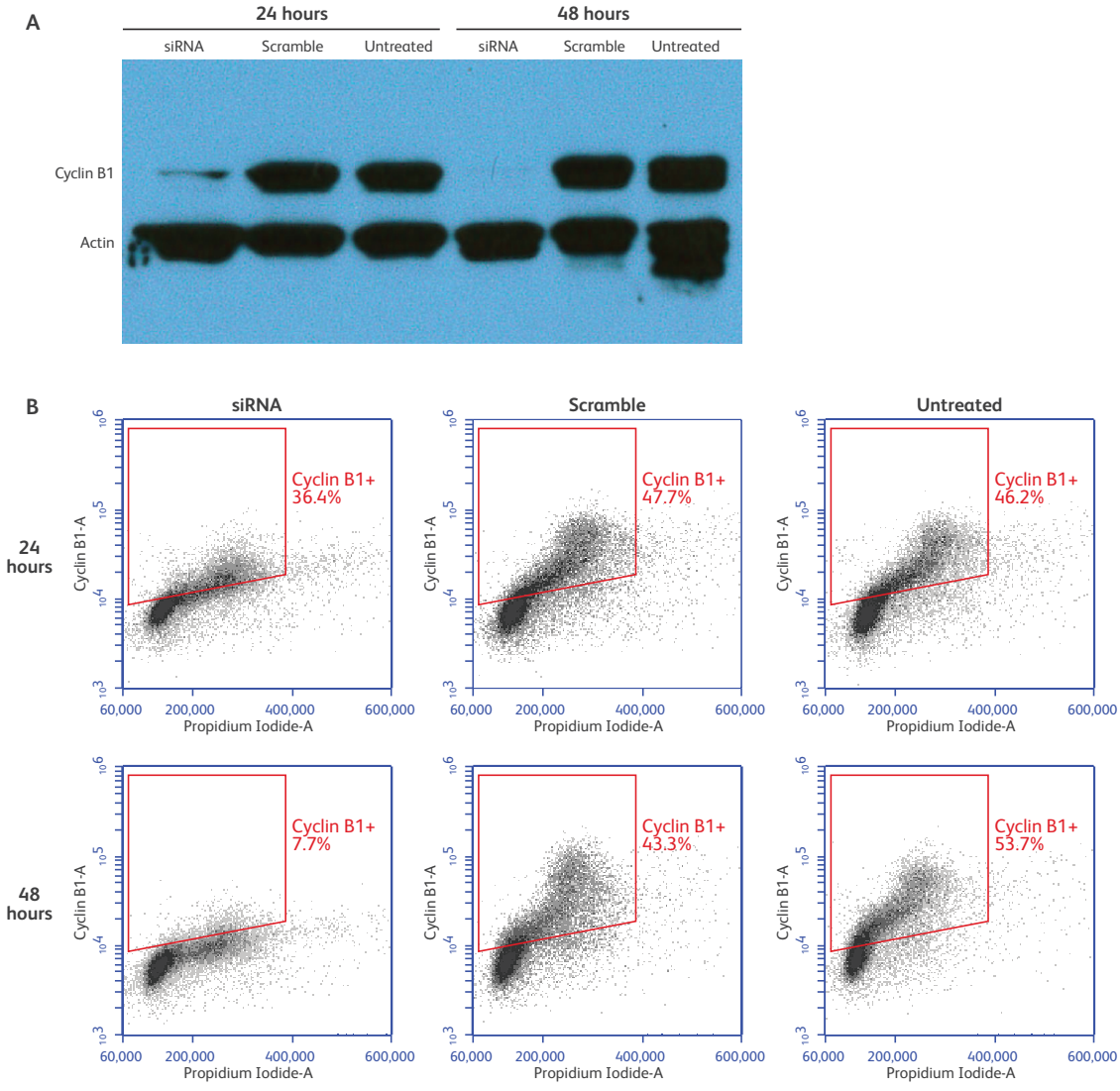

Figure 1. Monitoring cyclin B1 knockdown by Western blot and flow cytometry

HEK-293 cells were reverse transfected in 6-well plates using 5 µL of Lipofectamine® RNAiMAX (Thermo Fisher Scientific) per well and 30 pmol of cyclin B1 siRNA (siRNA), negative control scrambled siRNA (Scramble), or no siRNA (Untreated). After 24 or 48 hours, cells were harvested from culture and split for Western blot and flow cytometry analysis. **A. Western blot analysis** of cyclin B1 (upper band). Actin (lower band) was used as the loading control. Film was exposed for 10 minutes. A faint band was detected in the knockdown condition (siRNA) at 24 hours, and no band was detected at 48 hours. **B. Flow cytometry analysis.** Cells were fixed and permeabilized, and stained for cyclin B1 expression and DNA content (using propidium iodide). Data was collected and analyzed on a BD Accuri C6 Plus personal flow cytometer. All data was gated based on the light-scatter properties of single HEK-293 cells, and cyclin B1 expression was gated based on unstained controls. After 24 hours, the percentage of cells expressing cyclin B1, and the expression level within those cells, were reduced in siRNA-treated cells compared to scrambled control and untreated cells. After 48 hours, cyclin B1 expression was minimal in siRNA-treated cells.

Figure 2 demonstrates the ease and speed of optimizing a transfection protocol using the BD Accuri C6 Plus. A GFP expression vector was transfected into HeLa cells using different amounts of the transfection reagent. Since GFP can be detected by both cell imaging and flow cytometry, both techniques were used to assess transfection efficiency. The results from cell imaging (Figure 2A) indicated that using 1.50 µL of the transfection reagent provided the highest transfection efficiency—keeping in mind the caveat that traditional fluorescence microscopy for GFP⁺ cells relies on analysis of a limited number of cells fixed onto a slide and present in a few representative fields of view, as opposed to a more global assessment of all the cells in culture.

In contrast, flow cytometry-based analysis on the BD Accuri C6 Plus (Figure 2B) not only confirmed that 1.50 µL of transfection reagent was optimal for transfection (consistent with the cell imaging results), but also provided a quantitative assessment for the number of cells expressing GFP in each experimental condition tested. In addition, the enhanced sensitivity of flow cytometry allowed detection of a gradient of GFP-expressing cells stretching from left to right, corresponding to cell subsets containing different copy numbers of the GFP expression vector.

Easy to use, simple to maintain and affordable, the BD Accuri C6 Plus personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors and four fluorescence detectors. A compact and transportable design, fixed laser alignment, pre-optimized detector settings and automated instrument QC result in a system that is simple to use. For walkaway convenience, the optional BD CSampler™ Plus accessory offers automated sampling from 24-tube racks or multiwell plates.

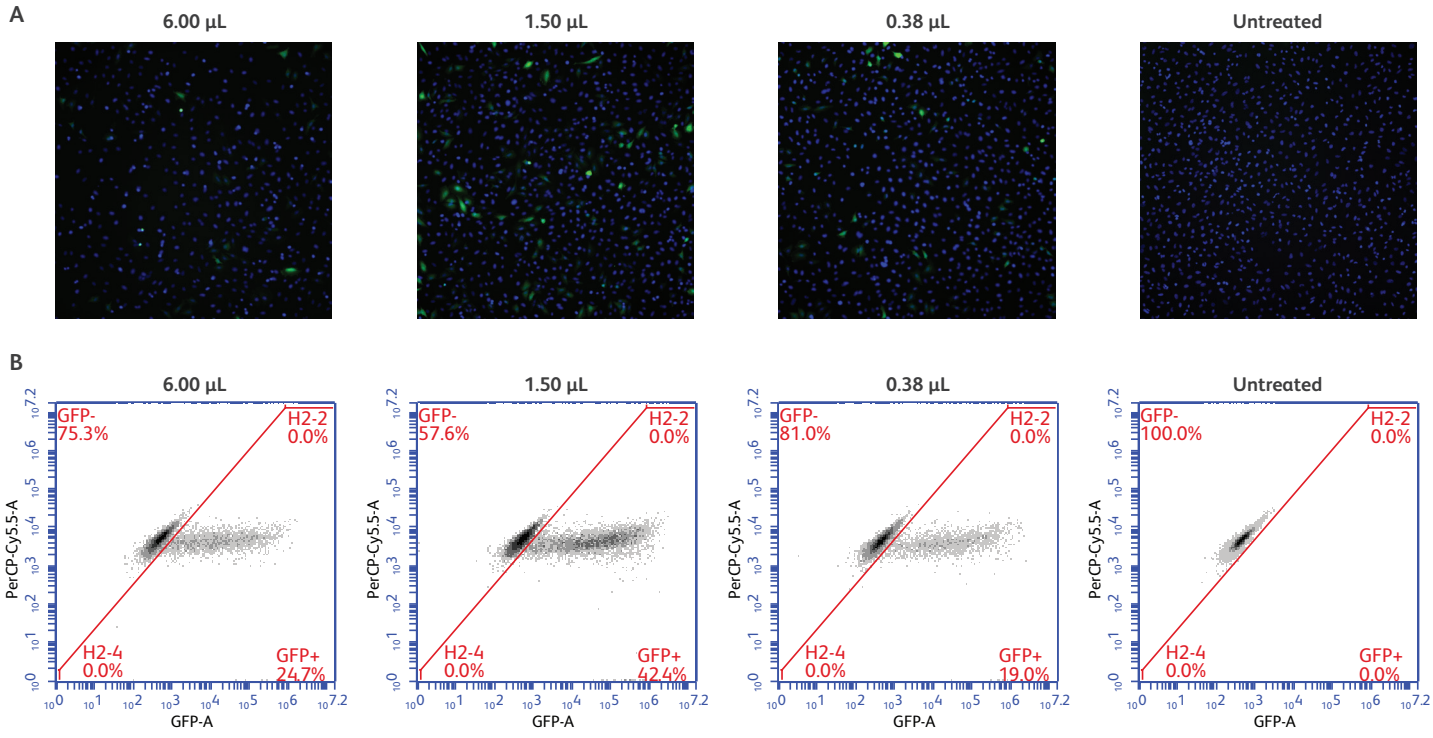

Figure 2. Monitoring transfection efficiency by imaging and flow cytometry

HeLa cells were plated at 2 x 10^5 cells/well in 6-well plates in antibiotic-free medium. The next day, the indicated amounts of X-tremeGENE™ 9 DNA Transfection Reagent (Roche) and 0.25 µg of vector pAcGFP-N1 (Clontech) were mixed and then added into the culture according to the manufacturers' recommendations. **A.** After 24 hours, cells were stained with 5 µg/mL of BD Pharmingen™ Hoechst 33342 Solution (pseudo-colored blue) and then imaged using an ImageXpress® Micro XLS widefield high-content analysis system (Molecular Devices). Higher expression of GFP⁺ cells (pseudo-colored green) was observed in the 1.50- and 0.38-µL conditions, and lower in the 6.00-µL condition. **B.** After fluorescent imaging, cells were harvested into a single-cell suspension in BD Pharmingen™ Stain Buffer (FBS) and then analyzed by flow cytometry using a BD Accuri C6 Plus. All data was gated based on the light-scatter properties of single HeLa cells. The PerCP-Cy™5.5 channel was used to distinguish dim GFP⁺ cells from autofluorescence. The combination of 0.25 µg of vector and 1.50 µL of transfection reagent per well had the highest transfection efficiency (42.4%) as assessed by the percentage of cells expressing GFP.

Ordering information

Description	Cat. No.
BD Accuri™ C6 Plus Flow Cytometer System	660517
BD Accuri™ C6 Plus Workstation and Software	661391
BD CSampler™ Plus Automated Sampling System (optional)	660519

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