Transfecting Small Molecules, Peptides & Proteins for Pharmaceutical Research

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Abstract

The Nucleofector® Technology enables efficient and reproducible transfection of primary cells and cell lines at throughputs of up to 96 samples per run.

Nucleofection® now extends its range of application to deliver small molecules substrates and protein substrates such as peptides, proteins and antibody-conjugates.

The Nucleofector 96-well Shuttle® System combines the proven advantages of Nucleofection with the flexible throughput capacity to enable cell-based screening approaches using RNAi or peptide libraries, early ADME-Tox applications using small molecules, or pathway validation using inhibitory peptides or antibodies.

The delivery of small molecules, peptides and antibodies is presented, demonstrating the capabilities of the Nucleofector Technology and its potential to enhance standard applications in pharmaceutical research and drug discovery.

Results

Using Nucleofector Technology, both cell-permeating and non-permeating compounds were successfully transfected. All cell types tested were efficiently loaded with substrate.

Transfection of small molecule substrates was homogenous and dependent on both the extra-cellular substrate concentration and the Nucleofection conditions used. Inhibitory small molecules were successfully transfected to cause cell-cycle arrest.

The Nucleofection of fluorescent substrate was shown to be effective for monitoring variations in transporter protein activity.

Nucleofection of non-membrane permeating, inhibitory peptides was used to suppress NF-κB - luciferase expression in a stable clone of HEK-293.

Conclusion

Nucleofection delivers substrates ranging from small molecules to antibody conjugates. This novel deliver method offers a valuable tool for:

› Delivery of non-cell permeating molecules

› Excretion kinetic analyses

› Target identification & validation

› Pathway analyses

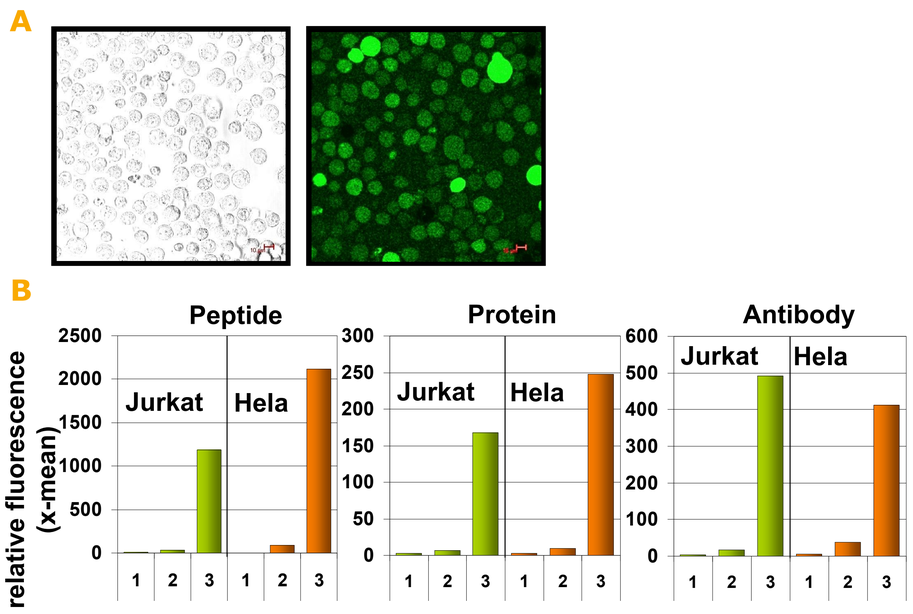

Figure 1: Different fluorescent substrates can be delivered into more than 90% of transfected cells.
A: Jurkat cells transfected with a fluorescently labelled peptide. Directly after substrate delivery cells were analyzed by light (left) and fluorescence microscopy (right).
B: Bar diagrams showing the transfection efficiency analysed by flow cytometry in Jurkat and Hela cells transfected with fluorescently labelled peptide, protein and antibody (1: ctrl, 2: substrate without Nucleofection, 3: Substrate with Nucleofection).

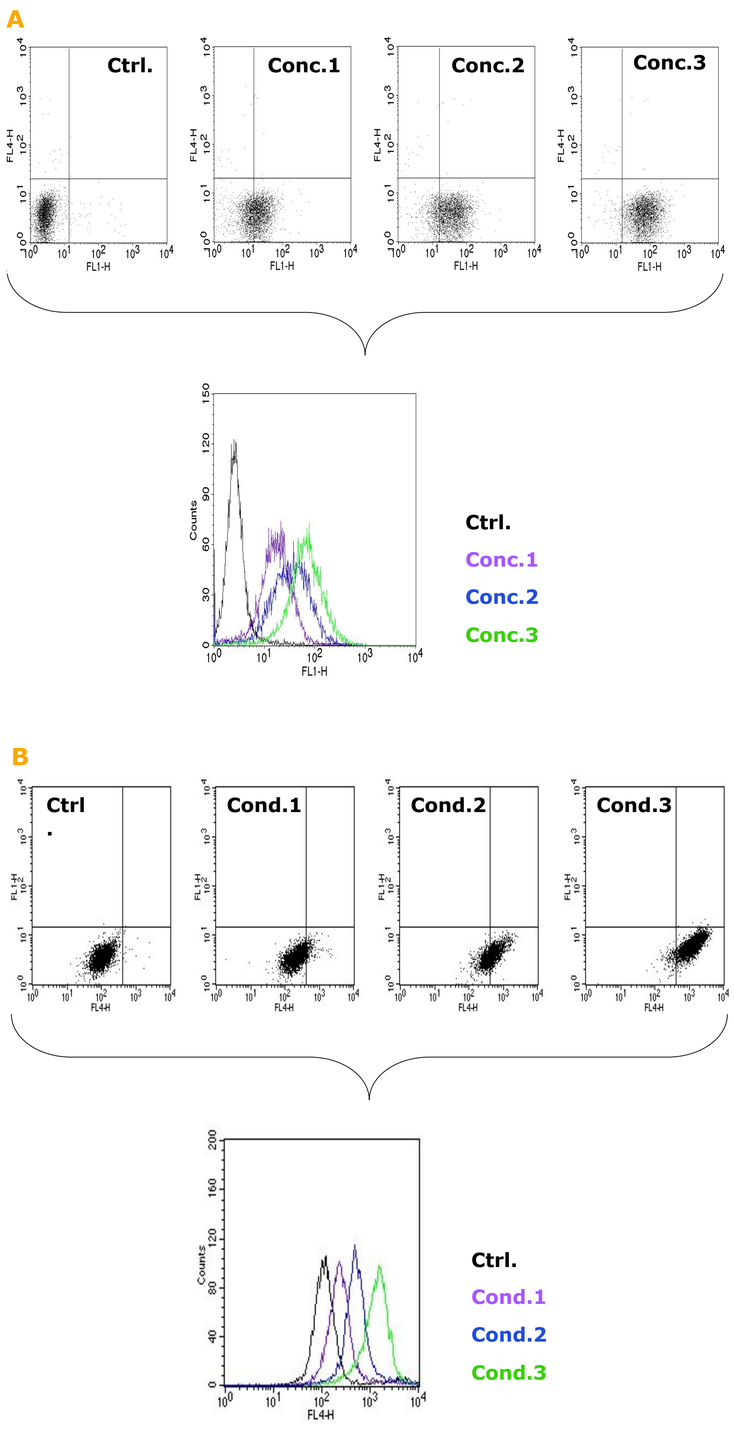

Figure 2: Compound delivery in K562 cells is homogenous and dose-and condition dependent. The fluorophore carboxyfluorescein was transferred into K562 cells. Cells were washed and analyzed directly after Nucleofection.
A: Dot plots and histogram plot of cells transfected by Nucleofection with carboxyfluorescein and analysed by flow cytometry showing that the intracellular concentration is dependent on the extracellular concentration used for Nucleofection.
B: Dot plots and histogram of cells transfected with carboxyfluorescein and analysed by flow cytometry showing that efficiency of delivery can be optimised or manipulated using alternative Nucleofection programs.

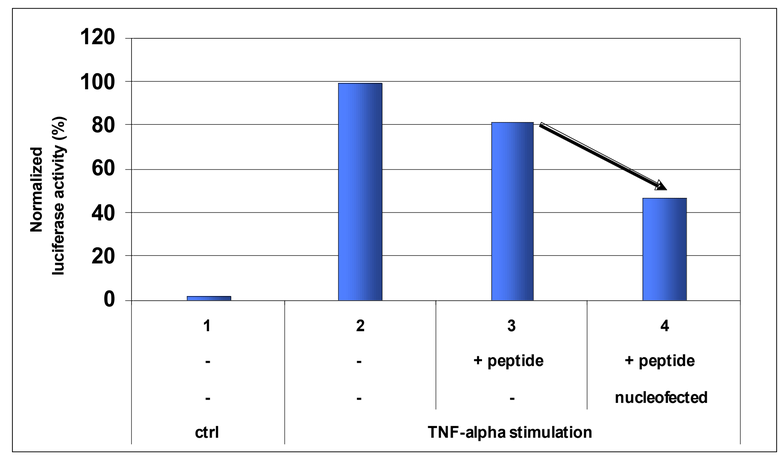

Figure 3. Specific inhibition of luciferase gene expression in a stable NF-κB-luciferase 293 cell line. NF-κB Reporter 293 cells (Panomics) stably expressing a reporter luciferase gene under the control of the transcription factor NF-κB exert luciferase activity via the NF-κB pathway when stimulated with TNF-alpha (see 1: no TNF-alpha stimulation; 2: TNF-alpha stimulation). Incubation with peptides, which interfere with this signalling pathway results only in a minor inhibition of the luciferase activity, probably due to low cell permeability of the peptides (see 3). Nucleofection of peptides results in a significant inhibition of luciferase activity by delivering peptide into the cell (see 4).

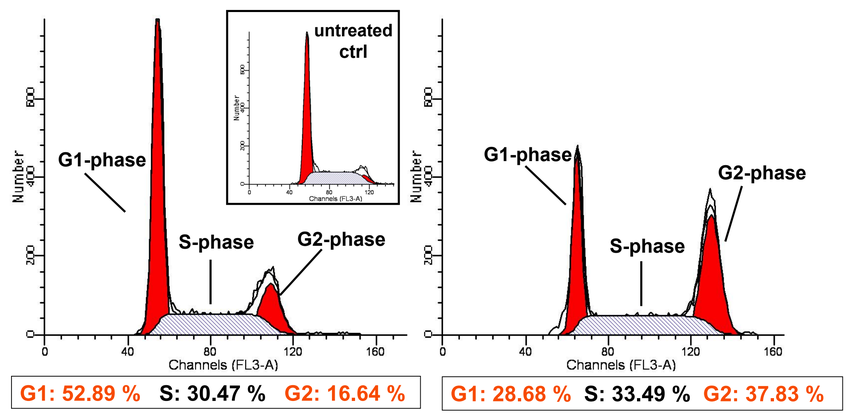

Figure 4: Higher Effectiveness in cell cycle arrest analysis in CA46 cells. CA46 cells (ATCC®, CRL-1648™) were resuspended in medium containing a defined concentration of a cell cycle kinase inhibitor. In parallel, cells were transfected at the same concentration prior to resuspension. A cell cycle analysis was performed by flow cytometry with PI/ RNase staining to determine G2-phase arrest. Without Nucleofection CA46 cells (left) show only a small increase in the G2-phase fraction. At the same concentration with Nucleofection the G2-phase fraction of CA46 cells is significantly increased (right).

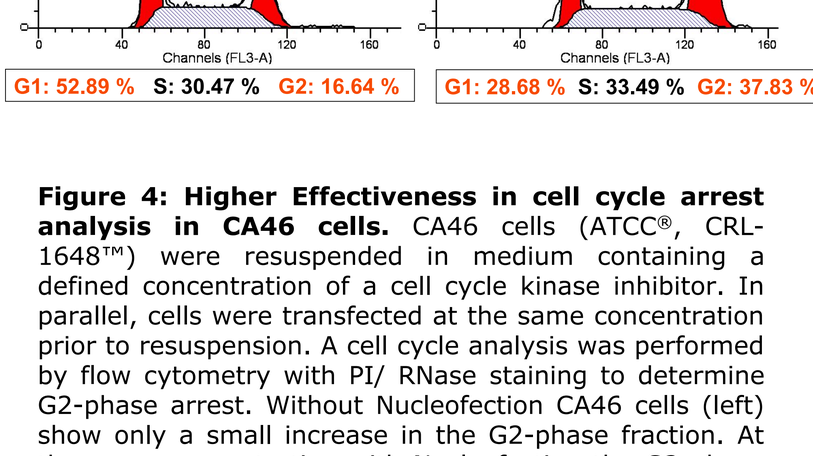

Figure 5: Characterization of efflux kinetics of wild type and a modified kidney cell line. A known fluorescent substrate for a transporter protein was transfected by Nucleofection into wild type cells (wt) and modified cells, which stably over express high amounts of transporter protein (modified). Within 1 hour the efflux of the fluorescent substrate was determined in a flow cytometer by means of the decrease of cell fluorescence. The export of the fluorescent substrate is accelerated in the modified kidney cell line.