

Amaxa[®] Nucleofector[®] Technology

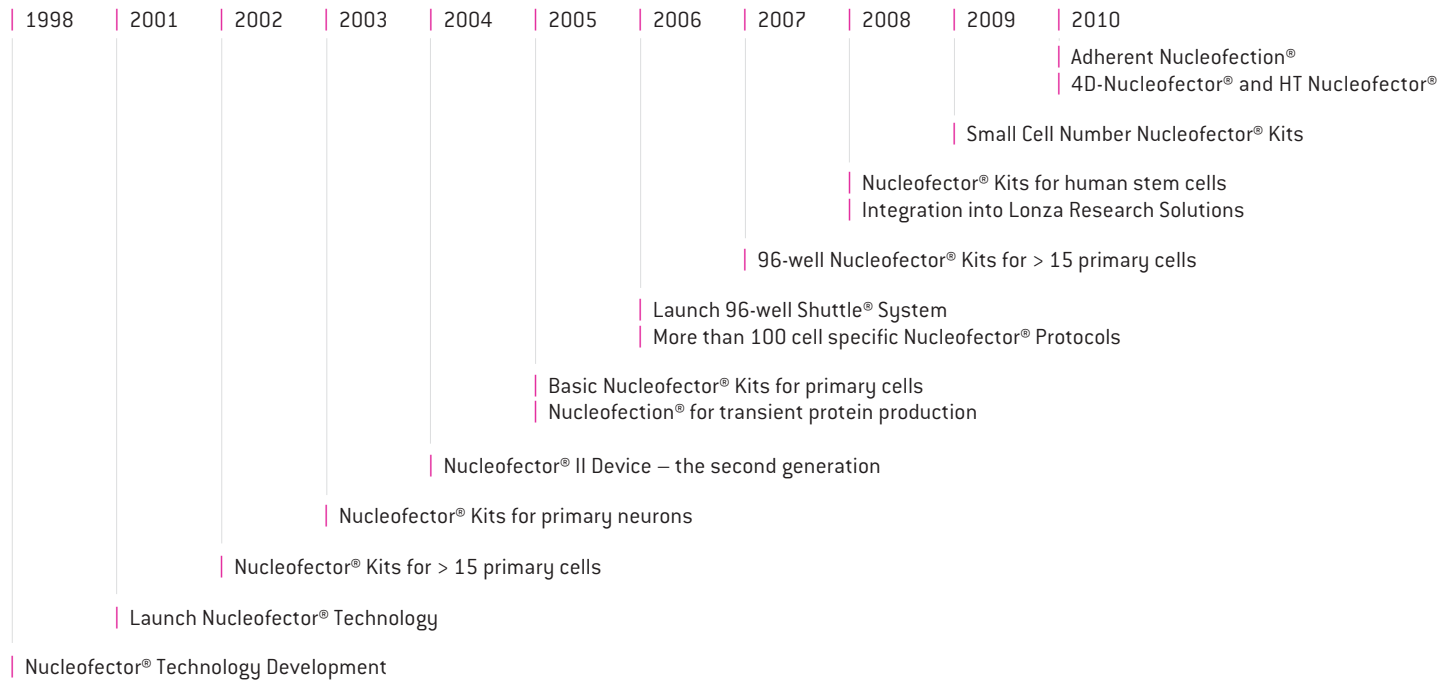
Do you have cells that are difficult-to-transfect?



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History of constant innovation



Introduction: Amaxa® Nucleofector® Technology

The application of systems biology and multidisciplinary approaches require that cells and model systems display *in vivo* like cellular functionality. This means that the future of cell transfection is in using primary cell types, and that transfecting these physiologically relevant cell types is typically a very difficult task using traditional methods. Additionally, when using relevant cell lines as model systems, the critical issues are to achieve reproducibly efficient transfection with high levels of viability while matching throughput capability with the number of transfections required at each project phase – from proof of concept, through to scale-up and screening-like approaches. With the **Amaxa® Nucleofector® Technology** primary cells and stem cells, as well as cell lines, can be consistently transfected at high efficiency.

Developed in 1998, the Amaxa® Nucleofector® Technology was introduced to the research market in 2001 as the first efficient non-viral transfection method for primary cells and hard-to-transfect cell lines. Since then the technology has evolved through constant innovation.

Now, 2010, the technology is lifted to the next dimension with its two new platforms:

- The 4D-Nucleofector® System offering highest flexibility for numerous transfection applications
- The HT Nucleofector® System enabling Nucleofection® in 384-well format

The Principle

Nucleofection® is a technology based on the momentary creation of small pores in cell membranes by applying an electrical pulse. The comprehensive way in which Nucleofector® Programs and cell type-specific solutions are developed enables nucleic acid substrates delivery not only to the cytoplasm, but also through the nuclear membrane and into the nucleus. This allows for high transfection efficiencies up to 99% and makes the transfection success independent from any cell proliferation.

DNA delivery straight into the nucleus

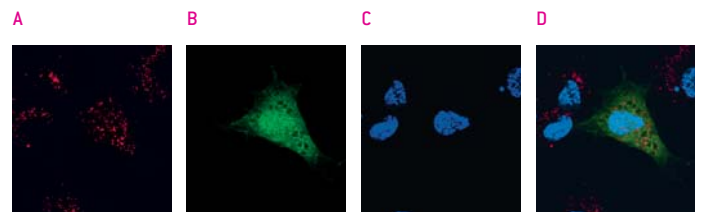


Figure 1: Normal human dermal fibroblasts (neonatal) were transfected with 2.5 µg TMR-labeled plasmid DNA encoding eGFP. After 2 hours, cells were fixed with 3.5% PFA and analyzed by confocal microscopy. TMR label is shown in (A), GFP fluorescence in (B), DAPI nuclear staining in (C) and a merge of all three fluorescent labels in (D).

Nucleofector® Technology – the superior non-viral method

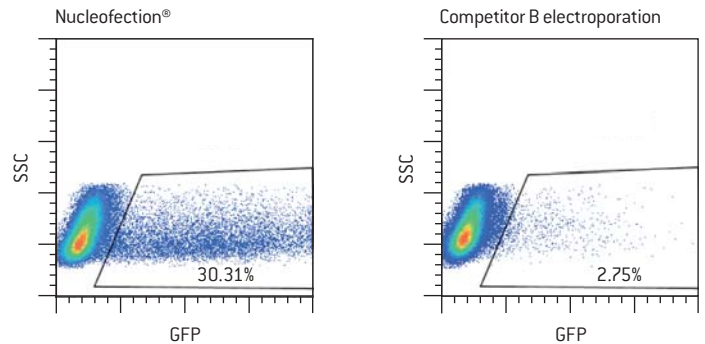


Figure 2. Transfection of the human natural killer cell line NK1 using traditional electroporation and Nucleofection®. 5×10^6 NK1 cells were transfected with 2.5 µg of pmaxGFP® Vector. Nucleofection®: Nucleofector® Solution V; Program 0-017. Competitor B electroporation: 25 mV, 96 µF. Transfection efficiency was monitored by flow cytometry after 24 hours. Cells transfected by Nucleofection® show a significantly better transfection efficiency compared to cells transfected by traditional electroporation. Cell viability, as measured 18 hours after transfection was also superior using Nucleofection®.

[Data courtesy of Dr. John Coligan, Laboratory of Immunogenetics, NIH/NIAID, Rockville, MD, USA. J Immunol Methods [2004] 284: 133-140.]

Average transfection efficiency for primary cells and human stem cells

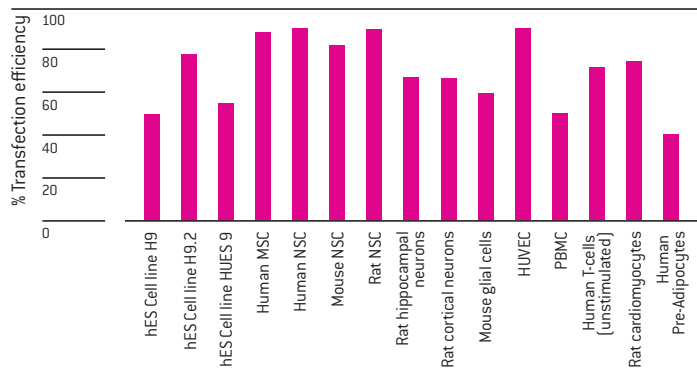


Figure 3. Overview about transfection efficiencies achieved by Nucleofector® for various primary cells and stem cells.

Conserving functionality – the first step to meaningful analysis

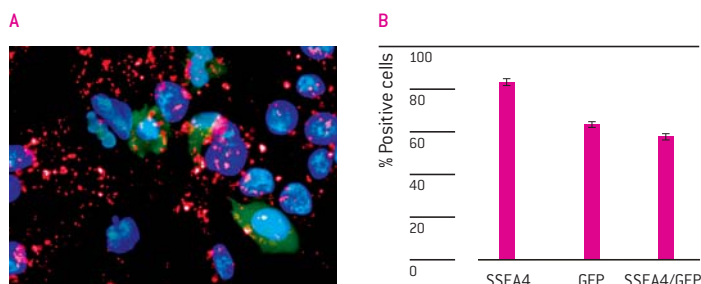


Figure 4. Human H9 ES cells preserve pluripotency post Nucleofector®. H9 cells were transfected by Nucleofector® with the pmaxGFP® Vector. (A) Cells analyzed after 24 hours show expression of GFP (green) as well as of the pluri potency markers SSEA4 (red) and Oct4 (purple). The blue signals refer to nuclear staining by DAPI. (B) The percentage of double-positive cells (GFP/SSEA) was analyzed by flow cytometry. (Data kindly provided by Jennifer Moore, Rutgers University, Piscataway, USA.)

Nucleofector® Technology – delivers the widest range of substrates

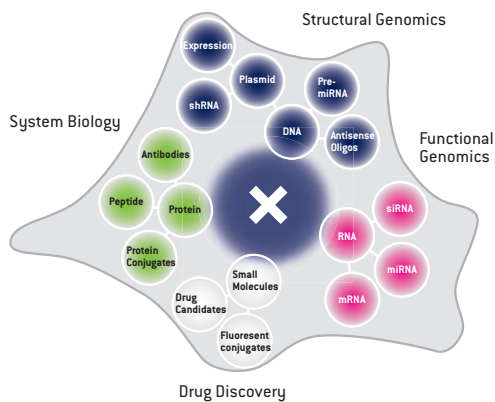


Figure 5. Overview of substrates that can be transfected into primary cells and cell lines using Nucleofector®.

What benefits are important for your work?

Looking for Superior Transfection Performance?

- Electrical parameters are optimized to gain high transfection efficiency and retain highest viability
- Excellent preservation of the physiological status of transfected cells

Easy-to-use technology?

- More than 650 cell-type specific protocols lead to direct transfection success with a multitude of different cell types
- Easy optimization protocols for cell lines and primary cells allow for quick and streamlined optimization of virtually any cell type
- Dedicated White Papers support numerous applications such as siRNA transfection and transfection of neurons

Excellent technical and applicative support?

- Highly-skilled scientific support team to assist you in your research
- Scientific Support Team members have a master or PhD level education in biology, biochemistry or biotechnology
- Many with over 10 years experience in transfection support

Relying on a proven and innovative technology?

- More than 4000 peer-reviewed publications and thousands of systems placed worldwide
- Modularity of the 4D-Nucleofector® System allows easy adaptation to new applications
- Invention of Nucleofector® of cells in adherence

Using various cell numbers for different applications?

- Nucleofector® of 2×10^4 to 2×10^7 cells are feasible within one single device
- Transferability of protocol conditions from small to larger cell numbers with the new 4D-Nucleofector® System

Easy expansion of your research?

- Explore complex systems by using the same conditions to deliver DNA, RNA, oligonucleotides, PNA, peptides, or proteins
- Different device platforms fulfill your choice of sample throughput from 1 through 384 transfections per run including automated high throughput

Avoiding cross-contamination?

- Disposable, sterile Nucleofector® Vessels minimize the risk of cross-contamination with cell or substrate leftovers

The Components of the Amaxa® Nucleofector® Technology

The Nucleofector® Technology relies on the combination of a Nucleofector® Device and cell specific Nucleofector® Kits.

The Nucleofector® Device

The Nucleofector® Device delivers unique electrical parameters. The electrical settings are pre-programmed for each optimized cell type and can be selected via the device or a PC software. We offer 3 different device platforms plus an add-on device:

- The new **4D-Nucleofector® System** is the most advanced platform offering greatest flexibility in terms of cell numbers and samples transfected, and it enables Nucleofection® of cells in adherence.
- The **96-well Shuttle® Device** is an add-on device compatible with the 4D-Nucleofector® System. It serves as a higher throughput option and as convenient tool for cell optimizations on the 4D-Nucleofector® Device.
- The new **HT Nucleofector® System** is an independent system which supports up to 384 Nucleofection® Reactions in parallel. Thus, it is perfectly suited for screening applications.
- The **Nucleofector® II Device** is the basic device for single cuvette applications.

The Nucleofector® Kits

Nucleofector® Kits contain a specific Nucleofector® Solution and Supplement, specified cuvettes, pipettes, and the pmaxGFP® Control Vector. All Nucleofector® Solutions provide a protective environment that allows for high transfection efficiency and cell viability, while helping to maintain physiologically relevant cellular functions. A collection of different Nucleofector® Kits is available and optimized for primary cells or cell lines.

Amaxa® Optimized Protocols

Besides providing optimal Nucleofection® Conditions, Amaxa® Optimized Protocols offer comprehensive guidance, including tips for cell sourcing, passage, growth conditions and media, and post transfection culture.



Overview about Nucleofection® Platforms

	Advanced Platform	96-well Add-On	High-Troughput Platform	Basic Device
Device	4D-Nucleofector® System	96-well Shuttle® Device	HT Nucleofector® System	Nucleofector® II Device
Unit				
Throughput (samples per run)	Low to medium (1-16)	Low to high (1-96)	High (384)	Low (1)
Reaction volume	20 µl + 100 µl	20 µl	20 µl	100 µl
Electrode material	Conductive polymer	Conductive polymer	Conductive polymer	Aluminum
Low cell numbers (20 µl)	2 x 10 ⁴ to 1 x 10 ⁶	2 x 10 ⁴ to 1 x 10 ⁶	2 x 10 ⁴ to 1 x 10 ⁶	–
High cell numbers (100 µl)	2 x 10 ⁵ to 2 x 10 ⁷	–	–	2 x 10 ⁵ to 2 x 10 ⁷
DNA Vector amount/sample	0.2 – 1 µg (20 µl) 1 – 5 µg (100 µl)	0.2 – 1 µg	0.2 – 1 µg	1 – 5 µg
siRNA amount/sample (concentration 2 nM – 2 µM)	0.2 – 200 pmol (100 µl) 0.04 – 40 pmol (20 µl)	0.04 – 40 pmol	0.04 – 40 pmol	0.2 – 200 pmol
Adherent Nucleofection® (20 µl Nucleocuvette® Strips)	✓	✓	–	–
Compatibility with 96-well Shuttle® Device	✓	–	–	–

NEW! The Advanced Platform: 4D-Nucleofector® System offering Multi-dimensional Flexibility

Based on numerous user feedback, Lonza engineers and scientists have developed the new innovative 4D-Nucleofector® System. This system is designed for maximum flexibility and enables Nucleofection® of cells in several formats combined with advanced performance and convenience.



Hardware and Software Components

The 4D-Nucleofector® System is a modular system comprising one **Core Unit** and the **X Unit** as first available functional unit. Core and X Unit can be assembled side by side or on top of each other. Due to its modular design the 4D-Nucleofector® System is extremely flexible in regard to the supported applications. The Operation Software allows to design and save individual experimental setups. Additionally, a PC editor is available that enables predefinition of experiments on a PC which can then be uploaded to the 4D-Nucleofector® Core Unit via the integrated USB port.

■ What benefits are important for your work?

Using different cell numbers for different applications?

- Same protocol for 100 µl and 20 µl transfection volume
- 100 µl Nucleocuvette® for high cell numbers up to 2×10^7
- 20 µl Nucleocuvette® Strip for low cell numbers down to 2×10^4

Working with various throughputs?

- Flexible throughput from 1 to 16 samples
- Parallel processing of one or two 100 µl Nucleocuvettes®
- Pre-programming of settings for up to 50 single 100 µl Nucleocuvettes® or one 20 µl Nucleocuvette® Strip
- Kit costs tailored to your throughput

Transfecting different primary cell types?

- Only 5 primary cell kits covering a broad range of primary cells
- New Primary Cell Optimization Kit for cells lacking an Optimized Protocol
- Easy optimization of a variety of cell lines using the 96-well Shuttle® Add-on Device

Preserving cell functionality?

- Adherent Nucleofection® of neurons at later developmental stages
- No release of metal ions due to conductive polymer electrodes



1 The Operation Software – Intuitive tool operating the 4D-Nucleofector® System

- Easy-to-use interface
- Predefined Nucleofection® Parameters and Experiments
- PC editor for predefinition of experiments

2 The Core Unit - Controlling the 4D-Nucleofector® System

- 5.7" foldable touch screen to operate the system
- Controls up to 5 functional units
- USB port for software update and data transfer
- Comprises USB and serial connectivity for the 96-well Shuttle® Device

3 The X Unit – Supporting Nucleofection® of various cell numbers in different formats

- Features positions for 20 µl Nucleocuvette® Strips and 100 µl single Nucleocuvette®
- Seamless transfer of conditions between different Nucleofection® Vessels
- Suited for Nucleofection® of cells in adherence in 16-well Nucleocuvette® AD Strips
- Comprises HV connectivity for the 96-well Shuttle® Device
- Electrically driven drawer for cuvette retainer

4D-Nucleofector® Kits Tailored to Your Needs

Same conditions for different cells numbers

The X Unit of the 4D-Nucleofector® System can handle two different Nucleocuvette® Vessels both composed out of the same **conductive polymer electrode material**:



NEW! Single 100 µl Nucleocuvettes®:

- Novel conductive polymer 100 µl cuvettes replacing former aluminum cuvettes
- For high cell numbers at low throughput (e.g. for biochemical applications or Western Blots)



16-well 20 µl Nucleocuvette® Strips

- Same strips as those assembled to a 96-well Nucleocuvette® Plate
- For low cell numbers at medium throughput (e.g. reporter gene assays, RNAi)

As the same electrode material is now used for 20 and 100 µl cuvettes, Nucleofection® Conditions are transferable between the different Nucleocuvette® Vessels offering maximum flexibility and convenience:

- Once the conditions are known for one format they can be easily transferred to the other format.
- Conditions are transferable between different throughput formats (4D-Nucleofector® System, 96-well Shuttle® Device and HT Nucleofector® System).
- Existing 96-well Shuttle® Protocols can be used with the 4D-Nucleofector® System.

Transferability between Nucleofection® Conditions between different formats

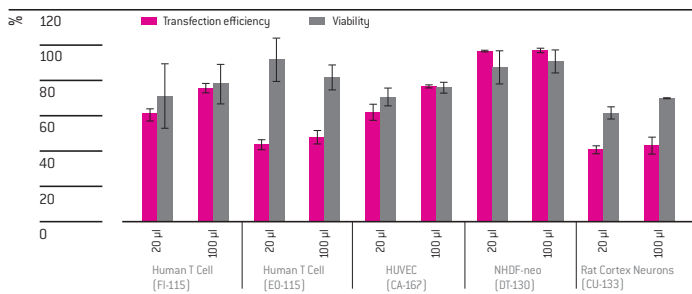


Figure 6. Various primary cells were transfected in the two Nucleocuvette® Vessel formats (20 µl and 100 µl) using the indicated programs. Twenty-four hours post Nucleofection® cells were analyzed for transfection efficiency (flow cytometry) and viability (cell number normalized to no program control).

Simplified primary cell kit concept

Conductive polymer electrodes were invented with the 96-well Shuttle® Device in 2006. Recent Lonza R&D findings have shown that with the conductive polymer technology less Nucleofector® Solutions are required to achieve optimal results for different primary cell types. In consequence, our R&D Team was able to narrow down the number of required 4D-Nucleofector® Solutions for primary cells to only 5 solutions which makes Nucleofection® of several different primary cell types much more convenient.

- A total of 5 dedicated Primary Cell 4D-Nucleofector® Kits, each suited for several primary cell types
- Primary Cell Optimization 4D-Nucleofector® Kit for optimization of primary cells lacking an Optimized Protocol

4D-Nucleofection® of cell lines

For transfection of cell lines using the 4D-Nucleofector® System the established Cell Line 96-well Nucleofector® Solutions SE, SF and SG are used.

Flexible kit formats and sizes

For the X-Unit of the 4D-Nucleofector® System different kit types are available in different formats and sizes – choose the optimal kit for your required cell number, throughput and budget:

4D-Nucleofector® Kits XS

- For Nucleofection® of lower cell numbers (from 2×10^4 to 1×10^6 cells)
- XS Kits contain 16-well Nucleocuvette® Strips (20 µl)
- Different kit sizes and volume discounts for different throughputs available

4D-Nucleofector® Kits XL

- For Nucleofection® of higher cell numbers (from 2×10^5 to 2×10^7 cells)
- XL Kits contain single Nucleocuvettes® (100 µl)
- Different kit sizes and volume discounts for different throughputs available

For more information please visit www.lonza.com/4d-nucleofector

4D-Nucleofector® System enabling Adherent Nucleofection®

Electroporation-based methods have so far required cells to be in suspension for transfection. The Nucleofector® Technology now enters a new era and allows direct Nucleofection® of cells in adherence. Cells derived from solid tissue (e.g. endothelial, epithelial or neuronal cells), which typically grow in adherence in cell culture, can be kept and transfected by Nucleofection® in their physiological state.

The **Basic Neuron Nucleofector® AD Kit** is the first of its kind that enables Nucleofection® of primary neural cells in adherence. The kit utilizes special 16-well Nucleocuvette® AD Strips (20 µl), a modified version of the currently available Nucleocuvette® Strips for Nucleofection® of cells in suspension. Different kit formats are available for various throughput requirements:

- 16-well format (for the 4D-Nucleofector® X Unit)
- 96-well format (for the 96-well Shuttle® Device)

Efficient Nucleofection® of Neurons in Adherence.

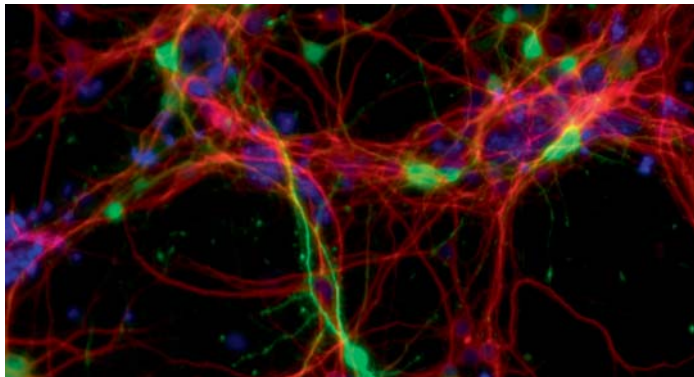


Figure 7. Mouse cortical neurons were isolated and seeded into poly-D-lysine coated 96-well Nucleocuvette® AD Plates at a density of 2×10^4 cells/well. After 4 days cells were transfected with 1 µg pmaxFP™-Yellow-C Vector using the Basic Neuron 96-well Nucleofection® AD Kit. Three days post Nucleofection® cells were stained with an anti-MAP2 antibody and DAPI and analyzed by fluorescence microscopy.

■ Benefit from an “all-in-one” solution:

- Pre- and post Nucleofection® culture for up to 14 days
- Nucleofection® of neurons at any time point during this culture period, i.e. at a later developmental stage
- High transfection efficiencies and viabilities
- Nucleocuvette® AD Modules suited for cell analysis by transmission light or fluorescence microscopy or by absorption, luminescence or fluorescence assays
- Compatible with Clonetics® Primary Animal Neurons

“All-in-one” Process

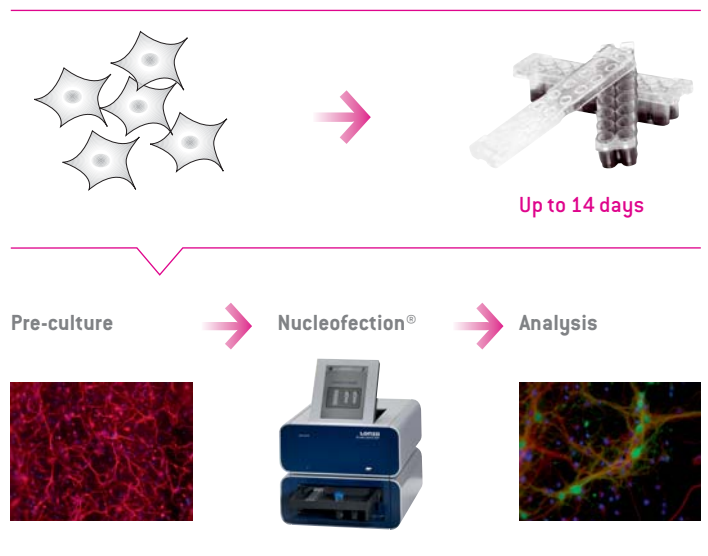


Figure 8. After isolation primary neurons are directly plated into poly-D- or poly-L-lysine coated Nucleocuvette® Strips. Cells can be cultured up to 14 days and transfected by Nucleofection® at any time during this period.

For the most recent information about Nucleofection® in adherence, please visit: www.lonza.com/adherent-nucleofection

The Add-On: 96-well Shuttle® Device



The 96-well Shuttle® Device delivers flexible throughput combined with economical processing, speed, and pre-optimized protocols for a range of both primary cells and cell lines. It is a medium throughput extension to the 4D-Nucleofector® Device suited for convenient optimization of Nucleofection® Conditions or as assay establishment tool. The complete system consists of three components:

- The 4D-Nucleofector® Device (Core and X Unit) serving as the program delivery unit
- The 96-well Shuttle® Device as contacting unit which mediates the transfer of the respective 96-well program to a specific well of the 96-well Nucleocuvette® Plate
- A laptop computer with the 96-well Shuttle® Software controlling the interaction between the devices

Consumables

The 96-well Shuttle® Device utilizes a unique disposable 96-well Nucleocuvette® Plate with conductive polymer electrodes which is part of each 96-well Nucleofector® Kit. The Nucleocuvette® Plate comprises 6 modules, each with 2 columns of 8 wells. Each Nucleocuvette® Well may be individually addressed with your pre-optimized program of choice. We offer a number of 96-well Nucleofector® Kits covering a broad range of primary cells and cell lines.

■ What benefits are important for your work?

Testing of various conditions for transfection or assay optimization?

- Up to 96 independent programs can be run per plate, processed automatically in less than five minutes
- Optimization of any difficult-to-transfect cell line in just one plate

Transfection with flexible throughputs?

- Modular 6 x 16 Nucleocuvette® Plate for scalable throughput
- Variable cell numbers – from 10⁴ – 10⁶ cells per reaction

Preserving cell functionality?

- Adherent Nucleofection® of neurons at later developmental stages
- No release of metal ions due to conductive polymer electrodes

Automated process required?

- Automatic processing of a 96-well plate in 3 – 4 minutes
- Fulfills prerequisites for liquid handling integration

Optimization of Nucleofection® Conditions within just one experiment

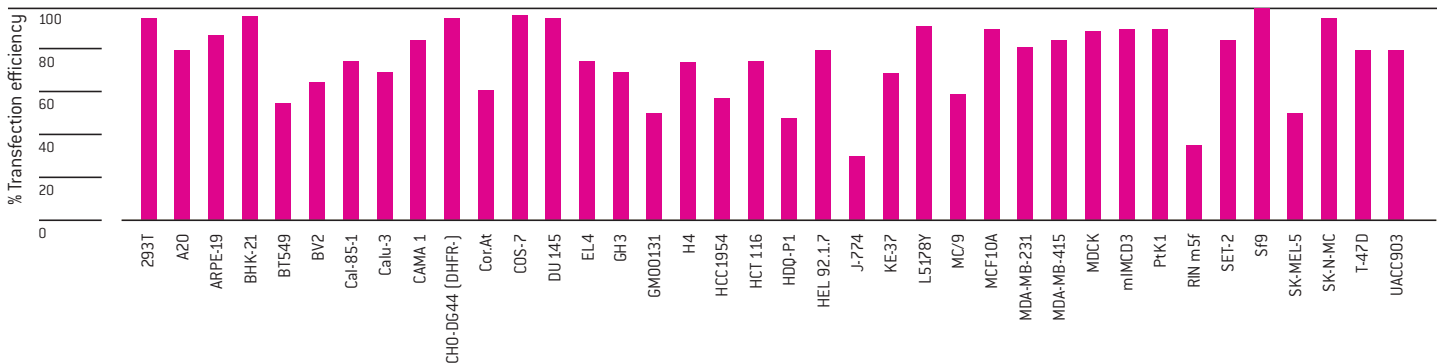


Figure 9. Examples of cell lines that have been optimized by customers using the Cell Line Optimization 96-well Nucleofector® Kit.

The Basic Device: Nucleofector® II Device



The Nucleofector® Device is the single cuvette based system that has been used in research labs since 2001. It allows efficient transfection of hard-to-transfect cell lines and primary cells with different substrates (e.g. DNA vectors or siRNA oligonucleotides) in low throughput format.

Consumables

- More than 50 dedicated primary cell kits, e.g. for blood cells or stem cells
- A collection of 5 cell line kits and an optimization kit covering the broadest range of cell lines
- cGMP kits for protein production applications

High transfection efficiencies in suspension cell lines.

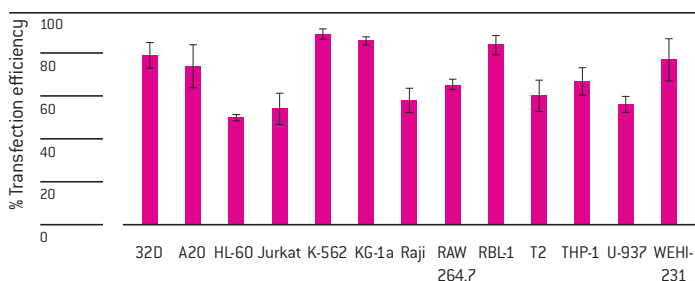


Figure 10. Transfection efficiencies 24 hours post Nucleofection® in selected cell lines relevant for immunology research. Cells were transfected with either eGFP, maxGFP® Reporter Protein or H-2K^k and analyzed 24 hours post Nucleofection®. Viability ranged from 60 – 90%.

■ What benefits are important for your work?

Highly efficient transfection in single cuvette format?

- Up to 90% efficiency with plasmid DNA
- Up to 99% efficiency with siRNA duplexes
- Also suited for peptides, proteins or small molecules

Easy-to-use technology with reliable results?

- More than 150 ready-to-use Amaxa® Optimized Protocols contain cell type-specific guidance
- Lanza's Cell Database contains user-developed protocols and data for more than 650 cell types
- Reliable and reproducible results due to high viability and preservation of cell functionality
- Approaching 4000 peer-reviewed publications

Consumables tailored to your needs?

- Nucleofector® Kits are available in low, normal and high usage format, preventing the waste of precious consumables and offer flexible pricing for different transfection throughputs

Nucleofector® Kits with aluminum cuvettes



NEW! The High-Throughput Platform: HT Nucleofector® System

The new HT Nucleofector® System is an independent platform for high-throughput Nucleofection® in 384-well format. With an extremely fast plate processing time of one minute it is perfectly suited for screening applications with maximum reproducibility. Furthermore cell storage time in Nucleofector® Solution is reduced to a minimum and all existing 96-well Shuttle® Protocols can be used without further optimization.

The components

The HT Nucleofector® System consists of a Power Supply Unit generating the high voltage pulses, a Plate Handler Unit (plate handling) and an intuitive PC-based Operation Software. The HT Nucleofector® Kits use existing 96-well Shuttle® Protocols but contain newly developed conductive polymer 384-well Nucleocuvette® Plates.

■ What is important for your screens?

Does speed count?

- Processes a 384-well plate in one minute
- Carousel handling two plates

Combining high performance with minimum material consumption?

- Nucleofection® of low cell numbers down to 2x10⁴ cells
- Option to adapt transfection volumes down to 5 µl

Easy-to-use and automatable system?

- Uses existing 96-well Shuttle® Protocols
- Operated by intuitive PC-software
- Seamless integration into automated liquid handling environments

The Plate Handler	The Power Supply	384-well Nucleocuvette® Plates	Operations Software – The Powerful Manager of the HT Nucleofector®
			
<ul style="list-style-type: none"> – Electrically driven carousel comprising two plate positions – Parallel loading and processing of plates – Small dimensions to occupy minimal space in automated environment 	<ul style="list-style-type: none"> – Can be positioned horizontally or vertically – Connected to Plate Handler via HV-cable/ data connection 	<ul style="list-style-type: none"> – Standard SBS format allows plate handling by automated liquid handling systems – No contamination of cell suspension with metal ions due to new conductive polymer cuvettes – Each well individually addressable (up to 384 conditions per plate) 	<ul style="list-style-type: none"> – Allows easy parameterization of HT Nucleofection® Experiments – Manages up to 384 different programs per plate (each well individually addressable) – Seamless integration into market leading liquid handling system – HT Nucleofector® Software Package including interface for LHS drivers

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