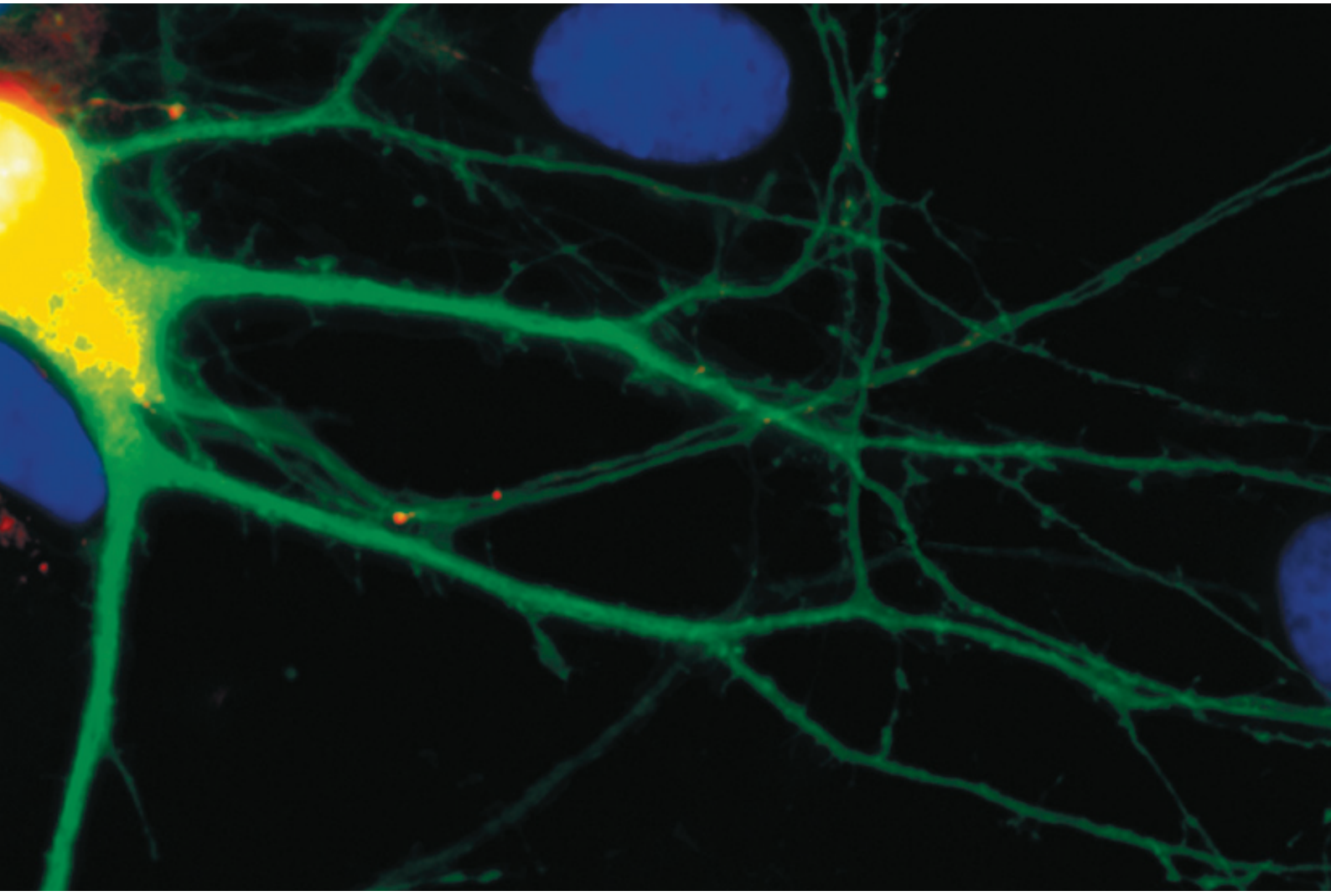
Lonza

Amaxa® Nucleofector® Technology – Maintenance of Functionality



Nucleofector® Technology – High Efficiency and Functionality

The Amaxa® Nucleofector® Technology has become the method of choice when transfection of primary cells or difficult-to-transfect cell lines is required. For some primary cells, transient transfection can be achieved by using classical transfection methods. However, hardly any of the classical transfection methods such as lipofection gain high transfection rates combined with low post-transfection mortality and a good preservation of cell-specific functionality. Methods using endosomal uptake can mediate interferon responses or deplete surface-exposed receptors. Such unintended phenotypes can bias results and seriously impair functionality such as differentiation or other responses to stimuli. With the Nucleofector® Technology, Lonza offers a superior transfection method unifying high transfection efficiencies, low cytotoxicity and preservation of functionality. Great care is taken during optimization of the Nucleofection® Protocols to minimize the effects of the transfection process on cell functionality. This includes the verification of cellular functionality utilizing frequently used cell-specific assays early in the developmental process.

■ The Nucleofector® Technology conserves cellular functionality due to:

- Minimal cytotoxicity – thanks to optimized pulses and cell-type specific Nucleofector® Solutions
- Careful optimization – conservation of morphology, phenotypic markers and differentiation potential
- Virus- and reagent-free transfection – no reagent toxicity or immunogenic effects

Normal morphology and expression of typical markers

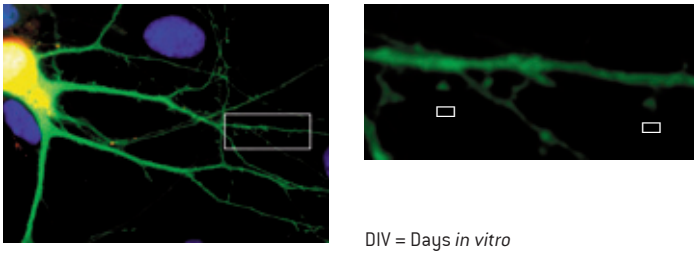


Figure 1: Normal neuronal development after 96-well Nucleofection®. P-0 neurons from rat hippocampus were transfected by Nucleofection® with pSyn-GFP and pDsRed and plated onto glass coverslips and examined after 7 days *in vitro* for GFP (green) and RFP (red) expression. Transfected neurons show an extensive dendritic network and, as shown in the magnification, develop dendritic protrusions that already resemble mature, mushroom-shaped dendritic spines (arrows). Surrounding, untransfected glia cells are shown by DAPI staining (blue).
(Data courtesy of Prof. Kiebler, Medical University of Vienna, Vienna, Austria.)

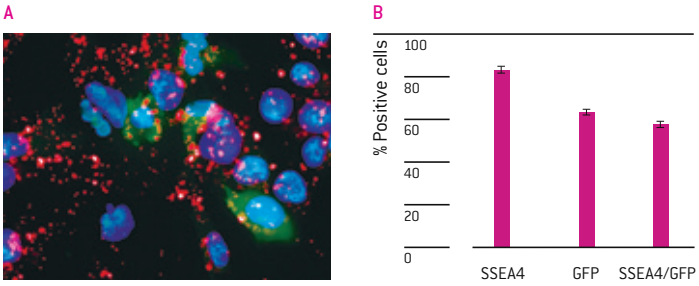


Figure 2: Human H9 ES cells preserve pluripotency post Nucleofection®. H9 cells were transfected by Nucleofection® with the pmaxGFP® Vector. (A) Cells analyzed after 24 hours show expression of GFP (green) as well as of the pluripotency markers SSEA4 (red) and Oct4 (purple). The blue signals refer to 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.)

Selected Publications

Hematopoietic cells	Cathelin S *et al.*, 2006 J Biol Chem 281(26): 17779-88	(Human monocytes)
	Landi A *et al.*, (2007) J Leukoc Biol 82(4): 849-60	(Human DC)
	Palmer CD *et al.*, (2008) Blood 111(4): 1781-8	(Human monocytes)
	Rebe C *et al.*, (2007) Blood 109(4): 1442-50	(Human monocytes)
Stem cells	Aluigi M *et al.*, (2006) Stem Cells 24(2): 454-461	(Human MSC)
	Aslan H *et al.*, (2006) Tissue Eng 12(4): 877-889	(hMSC; tissue regeneration)
	Cesnulevicius K *et al.*, (2006) Stem Cells 24(12): 2776-91	(Rat NSC)
	Hohenstein KA *et al.*, (2008) Stem Cells 26(6): 1436-1443	(Human ES cells)
Other	Noguchi M *et al.*, (2007) J Biol Chem 282(40): 29574-83	(3T3-L1 adipocytes)

For a most current overview or more detailed information, please see www.lonza.com/functionality.

Unaltered Differentiation Potential Post Nucleofection®

Maintainence of 'normal' cell phenotypes is important in most cell-based experimental systems, but is especially important for developmental research in embryonic and somatic cells and for hematopoietic cell research. Nucleofection® offers the ability to monitor undisturbed cell phenotypes and to transfect progenitor cells and monitor differentiation after transfection. For example, mesenchymal stem cells may be transfected using Nucleofector® Technology and then differentiated to osteoblastic, chondrogenic or adipogenic lineages.

Post-transfection differentiation

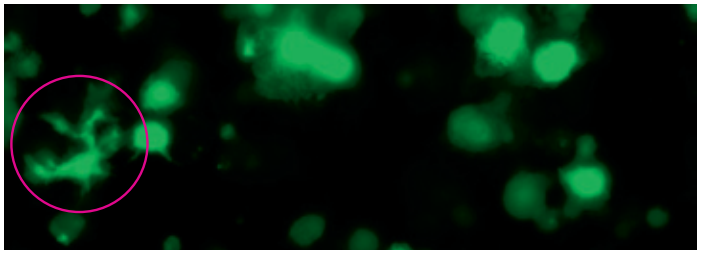
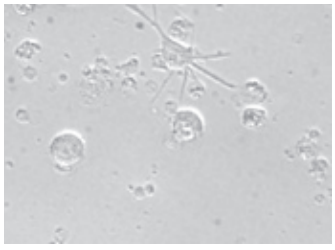


Figure 3: Maturation of mouse dendritic cells post Nucleofection®. Immature mouse dendritic cells were transfected with the pmaxGFP® Vector using the Mouse Dendritic Cell Nucleofector® Kit. 2 hours post Nucleofection®, cells were stimulated with LPS to mature. Cells were analyzed 24 hours post Nucleofection® by fluorescence microscopy for maxGFP® Reporter Protein expression and their ability to form dendrites (red circle).

A



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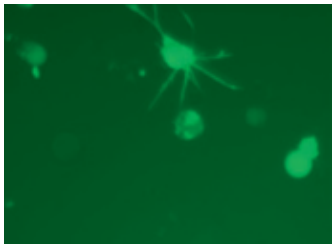

Figure 4: Differentiation of U937 cells into adherent macrophage-like cells post Nucleofection®. U937 cells (ATCC® CRL-1593.2™) were transfected with 2 µg pmaxGFP® Vector according to the optimized Nucleofection® Protocol. Immediately post-transfection, 100 nM PMA was added to the culture medium to induce differentiation. Cells analyzed after 72 hours by light (A) and fluorescence (B) microscopy were adherent (indicating differentiated status) and showed maxGFP® Reporter Protein expression levels higher than in non-differentiated cells.

No Induction of Interferon Responses

Lipid-based delivery reagents can cause cytotoxicity and are alone or in combination with double-stranded RNA capable of inducing a potent interferon response and/or altering gene expression profiles (reviewed in: Svoboda P, 2007, Curr Op Mol Ther 9(3): 248-257). These unintended phenotypes can significantly affect experimental outcomes and drastically interfere with the specificity of RNAi experiments. A described trigger of interferon responses when transfected with lipofection does not induce typical responses, such as upregulation of the interferon-sensitive OAS-1 gene or an increase in IL-6 release, when transfected by Nucleofection® (Figure 5).

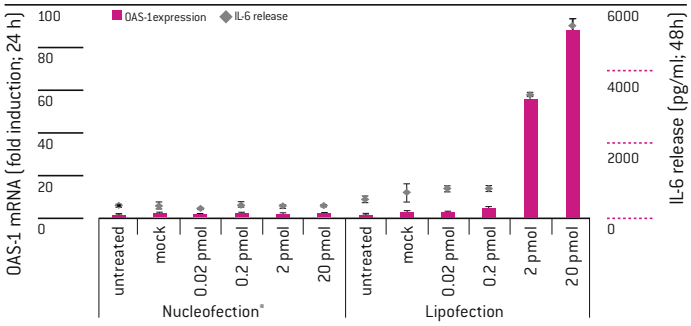

Figure 5: Induction of interferon responses is related to the method of substrate delivery. HeLaS3 (ATCC® CCL-2.2™) were transfected with different amounts of 29-mer siRNA targeting DBI (Dharmacon) – a described trigger of interferon responses (Reynolds A *et al.*, (2006), RNA 12:988-993) – using the 96-well Shuttle® Device or a lipid reagent. Interferon response was measured by expression of OAS-1 mRNA, determined 24 hours post-transfection by QuantiGene® Branched DNA Assay (Panomics; normalized to GAPDH mRNA), and IL-6 release analyzed 48 hours post-transfection by human IL-6 ELISA (Biosource).
(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products.)

Ordering Information

Cat. No.	Description
AAD-1001S	Nucleofector® II Device
AAM-1001S	96-well Shuttle® Device*

Standard and 96-well Nucleofector® Kits for Primary Cells**

For an up-to-date list of all standard and 96-well Nucleofector® Kits for Primary Cells please visit www.lonza.com/nucleofection-for-primary-cells.

Standard and 96-well Nucleofector® Kits for Cell Lines**

For an up-to-date list of all standard and 96-well Nucleofector® Kits for Cell Lines please visit www.lonza.com/nucleofection-for-cell-lines.

* Includes Laptop with Nucleofector® 96-well Shuttle® Software. To use the 96-well Shuttle® System, the Nucleofector® II Device is required.

** These kits contain a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as positive control with these Lonza products only. Any use of proprietary nucleic acid or fluorescent protein other than as positive control with this Lonza product is strictly prohibited. Use in any other application requires License from Evrogen. To obtain such a license, please contact Evrogen at license@evrogen.com.
The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

Lonza

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