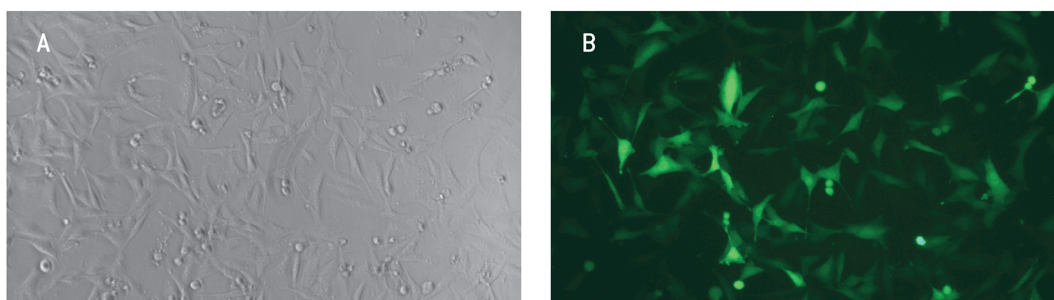
Lonza

Amaxa® Cell Line Nucleofector® Kit V

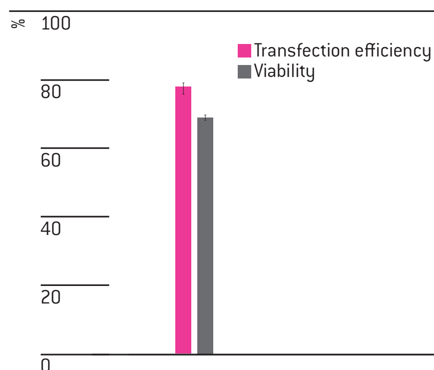
For T/C-28a2

SV40- immortalized human juvenile costal chondrocytes
polygonal / fibroblast like adherent cells

Example for Nucleofection® of T/C-28a2



T/C-28a2 cells were transfected using the Cell Line Nucleofector® Kit V, program X-001 (for Nucleofector® II) or X-01 (for Nucleofector® I) and 2 µg of pmaxGFP®. 24 hours post Nucleofection® the cells were analyzed by light (A) and fluorescence microscopy (B). Data kindly provided by Jochen Haag, University of Leipzig



Average transfection efficiencies and viability of T/C-28a2 cells. Cells were transfected with program X-001 (for Nucleofector® II)/X-01 (for Nucleofector®I) and 2 µg of pmaxGFP® Vector. 24 hours post Nucleofection®, the cells were analyzed by flow cytometry. Cell viability was analyzed 24 hours post Nucleofection® by using the CellTiterGlo assay (Promega). Data kindly provided by Jochen Haag, University of Leipzig.

Product Description

Cat. No.	**VCA-1003**
Size (Reactions)	25
Cell Line Nucleofector Solution V	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
Storage and stability	Store Nucleofector® Solution, Supplement and pmaxGFP® Vector at 4°C. For long-term storage, pmaxGFP® Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector® Supplement is added to the Nucleofector® Solution, it is stable for three months at 4°C.

Required Material

Note **Please make sure that the entire supplement is added to the Nucleofector® Solution.**

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260 : A280 ratio should be at least 1.8
- 6-well culture dish or culture system of your choice
- **For trypsinization:** 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- Appropriate volume of culture media at 37°C and 5% CO_2 (DMEM/Ham's F-12 supplemented with 10% FCS)
- Appropriate number of cells ($1x10^6$ cells per sample)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Passage cells every 4 – 6 days, split ratio 1 : 10

1.2 Seed **$1 x 10^6$** cells/ml per 10 cm dish 1 day before Nucleofection® to achieve the 80% confluence recommended for Nucleofection®

Note **Do not let the cells overgrow.**

Trypsinization

1.3 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media

1.4 For harvesting, incubate the cells 10 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)

1.5 Stop trypsinization reaction with DMEM/F12 supplemented with 10% FCS

2. Nucleofection®

One Nucleofection® Sample contains
1 x 10^6 cells
1 – 2 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 nM – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Nucleofector® Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 6-well plates by filling appropriate number of wells with 1.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Harvest the cells by trypsinization (please see 1.4 – 1.6)
2.4 Count an aliquot of the trypsinized cells and determine cell density
2.5 Centrifuge the required number of cells (**1 x 10^6 cells per sample**) at **300xg for 10 minutes** at room temperature
2.6 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
2.7 Combine 100 µl of cell suspension with **1 – 2 µg DNA or 2 µg pmaxGFP® Vector** or appropriate amount of **siRNA** (**30 nM – 300 nM** or 3 – 30 pmol/sample) or other substrates
2.8 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles
2.9 Select the appropriate Nucleofector® Program **X-001**
2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.11 Take the cuvette out of the holder once the program is finished
2.12 Add 500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the 6-well plate (final volume 1.5 ml media per well/sample). Use the supplied pipettes and avoid repeated pipetting of the sample

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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References

1. Mary B. Goldring et al. (1994) J. Clin. Invest. 94:2307-2316

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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

The Nucleofector® Technology, comprising Nucleofection® Process, Nucleofector® Device, Nucleofector® Solutions, Nucleofector® 96-well Shuttle® System and 96-well Nucleocuvette® plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne AG.

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