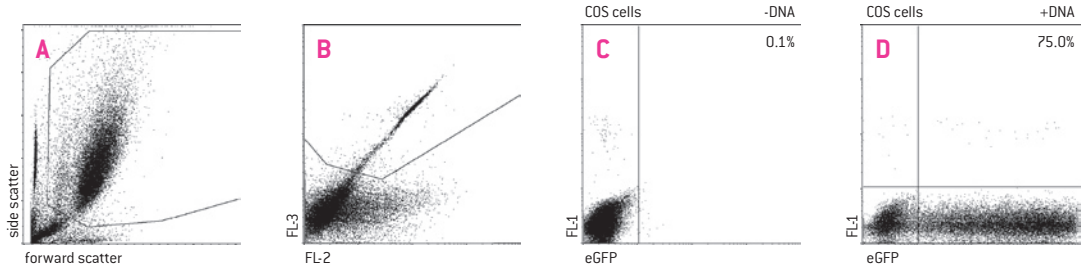
Amaxa® Cell Line Nucleofector® Kit R

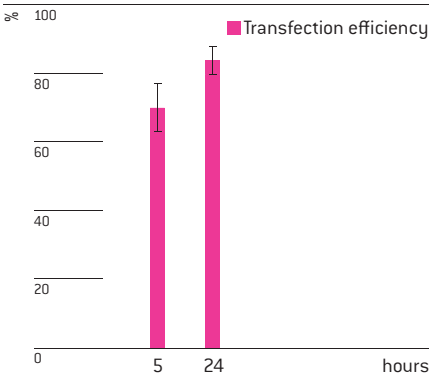
For NIH/3T3 (ATCC® CRL-1658™)

NIH Swiss mouse embryo; adherent fibroblastoid cells

Example for Nucleofection® of NIH/3T3 (ATCC® CRL-1658™)



NIH/3T3 cells (ATCC® CRL-1658™) were transfected with the Nucleofector® Kit R, Program U-030 and a plasmid encoding the enhanced green fluorescent protein eGFP. 5 hours post Nucleofection®, the cells were analyzed by light (A) and fluorescence microscopy (B).



Average transfection efficiency of NIH/3T3 cells. NIH/3T3 cells (ATCC® CRL-1658™) were transfected with program U-030 and 3 µg of plasmid encoding the enhanced green fluorescent protein eGFP. 5 and 24 hours post Nucleofection®, the cells were analyzed by flow cytometry.

Product Description

Cat. No.	**VCA-1001**
Size (reactions)	25
Cell Line Nucleofector® Solution R	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. The ratio of Nucleofector® Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- 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 detaching cells:** 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS
- **Culture medium:** 90% Dulbecco's modified Eagle medium (DMEM) with 4 mM L-glutamine, 1.5 g/l sodium bicarbonate and 4.5 g/l glucose [ATCC®, Cat; No. 30-2002] and 10% Bovine Calf Serum [ATCC®; Cat. No. 30-2030]. The medium and the FBS have a tremendous influence on both the transfection efficiency and cell survival of this cell line. We strongly recommend to use medium and FBS from ATCC® for culture and Nucleofection® Experiments
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10^6 cells per sample ; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media every 2 days
1.2 Passage cells at 70 – 80 % confluency. The cell layer should not become completely confluent
1.3 Seed out 10^2 – 10^3 cells/cm^2
1.4 Cells should be passaged 2 – 3 days before Nucleofection®
1.5 Optimal confluency for Nucleofection®: 80 – 90%. Higher cell densities may cause lower Nucleofection® Efficiencies

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with PBS
1.7 Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA
1.8 Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density

2. Nucleofection®

One Nucleofection® Sample contains

1 x 10^6 cells
1 – 5 µg plasmid DNA (in 1 – 2 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (0.6 – 6 pmol/sample)
100 µl Cell Line Nucleofector® Solution R

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 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 1.7 – 1.8)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (1 x 10^6 cells per sample) at 200xg for 10 minutes at room temperature. Remove supernatant completely
2.6 Resuspend the cell pellet carefully in 100 µl room-temperature Nucleofector® Solution per sample

Note **Avoid leaving the cells in Nucleofector® Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability.**

2.7 Combine 100 µl of cell suspension with 1 – 5 µg DNA, 2 µg pmaxGFP® Vector or appropriate amount of siRNA (30 nM – 300 nM or 0.6 – 6 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). Close the cuvette with the cap
2.9 Select the appropriate Nucleofector® Program **U-030** (U-30 for Nucleofector® I Device)
2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.11 Take the cuvette out of the holder once the program is finished
2.12 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6-well plate (final volume 1.5 ml media per well/sample). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

3.1 Incubate the cells in 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. Martinez-Gonzalez J et al., Circ Res. 2003;92(1):96-103

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