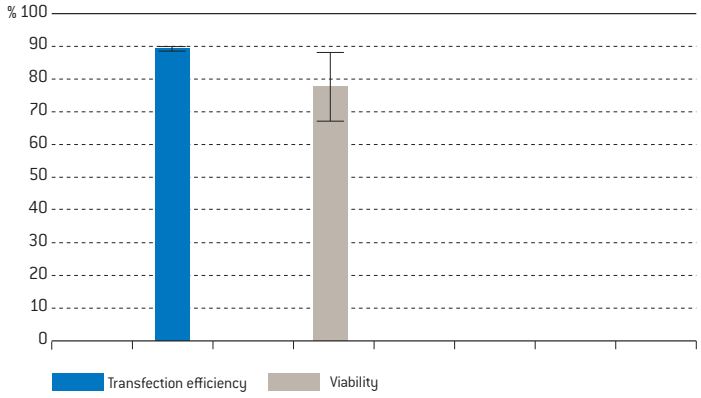
BioResearch

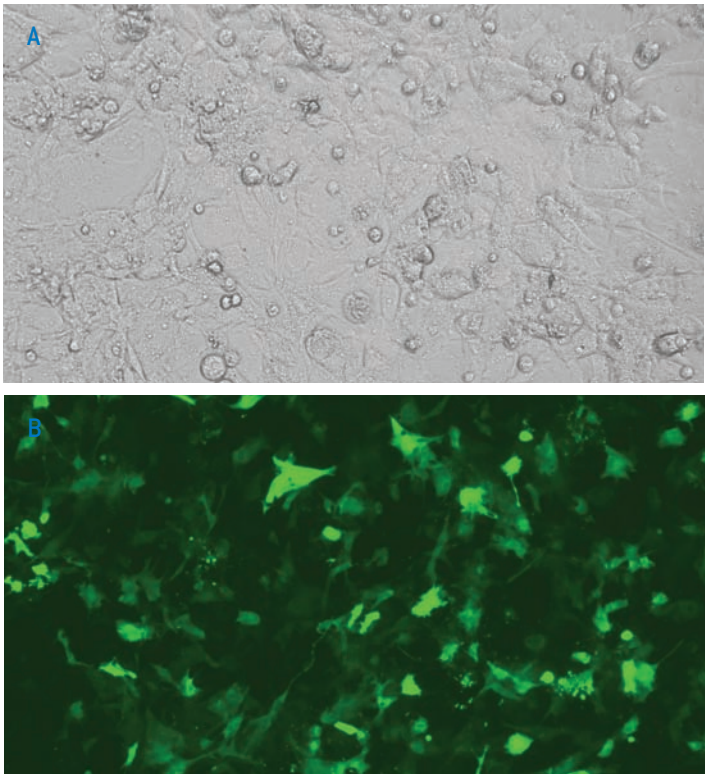
Lonza

Amaxa™ Nucleofector™ protocol for mouse embryonic fibroblasts (MEF; Lonza)

Example for Nucleofection™ of MEF cells



Average transfection efficiency of mouse embryonic fibroblasts. 2 x 10^6 cells were transfected with program N-024 using the pmaxGFP™ vector. cells were analyzed 24 hours post Nucleofection™ by flow cytometry. cell viability was measured with the ViaLight™ Plus Bioassay Kit; Lonza, Cat. No. LT07-221).



Primary mouse embryonic fibroblasts were transfected using pmaxGFP™ vector. cells were analyzed 24 hours post Nucleofection™ using light (A) and fluorescence microscopy (B).

Cell description

Validated to work with primary (non-immortalized) mouse embryonic fibroblasts from Lonza (Cat. No. M-FB-481). Fibroblastoid adherend cells.

Product description–Recommended kit(s)

Cat. No.	VPL-1004
Size	25 reactions
Mouse/rat hepatocyte Nucleofector™ solution (2.05 ml + 10% overfill) supplement (0.45 ml + 10% overfill)	2.25 ml 0.5 ml
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.

Note

During the optimization experiments it turned out that the mouse/rat Nucleofector™ solution is the optimal Nucleofector™ solution for transfection of primary MEF cells (Lonza; Cat. No. M-FB-481). For transfection experiments use the mouse/rat Nucleofector™ solution and follow this optimized protocol (Amaxa™ Nucleofector™ protocol for mouse embryonic fibroblasts). If you would like to transfect immortalized MEF please refer to our „MEF starter Nucleofector™ kit“ (Cat.No. VPD-1006) and the respective protocol.

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, software requirements: version V1.9 or higher for Nucleofector™ I Device; version S3.2 or higher for Nucleofector™ II 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: HEPES (CC-5022), Trypsin-Versene Mixture (17-161E) and Trypsin Neutralizing Solution (TNS) [Lonza, Cat. No. CC-5002]
- **Culture medium:** DMEM with 4.5 g/l glucose and 4 mM glutamine [Lonza; Cat. No. 12-604F] supplemented with 10 % fetal bovine serum (FBS) (Lonza; Cat. No. 14-503E), 100 µg/ml streptomycin and 100U/ml penicillin
- Prewarm appropriate volume of culture medium to 37°C (2.0 ml per sample)
- Appropriate number of cells: 2 x 10^6 cells per sample; lower or higher cell numbers may influence transfection result

1. Pre Nucleofection™

Note

Transfection results may be donor- or lot-dependent.

cell culture recommendations

1.1 Seeding conditions: 8–10 x 10^3 cells/cm^2

1.2 Cells should be passaged after reaching 70–80% confluency; replace media every two days

1.3 Optimal confluency before Nucleofection™ is 60–70%

1.4 Do not use cells after passage number 4 as this may result in substantially lower gene transfer efficiency and viability

Trypsinization

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

1.6 Cells are very sensitive to trypsin treatment. For harvesting, incubate the cells 1–3 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)

1.7 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached. Do not incubate the cells in TNS longer than 10 minutes

2. Nucleofection™

One Nucleofection™ sample contains

- 2 x 10^6 cells
- 1–5 µg plasmid DNA (in 1–5 µl H_2O or TE) or 2 µg pmaxGFP™ vector
- 100 µl Mouse/Rat Hepatocyte 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 2 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 1.5–1.7)

2.4 Count an aliquot of the cells and determine cell density

2.5 Centrifuge the required number of cells (2 x 10^6 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 and gene transfer efficiency

2.7 Combine 100 µl of cell suspension with 1–5 µg DNA, 2 µg pmaxGFP™ vector or 30 nM–300 nM siRNA (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). Close the cuvette with the cap
2.9 Select the appropriate Nucleofector™ program N-024 (N-24 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 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 2.0 ml media per well. Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection™

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

Up-to-date list of all Nucleofector™ Rreferences

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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