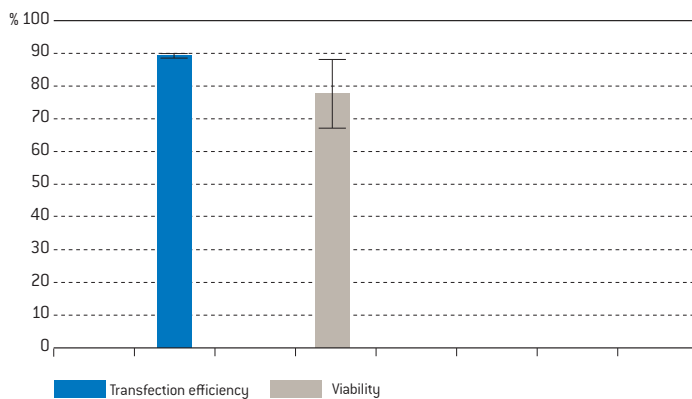
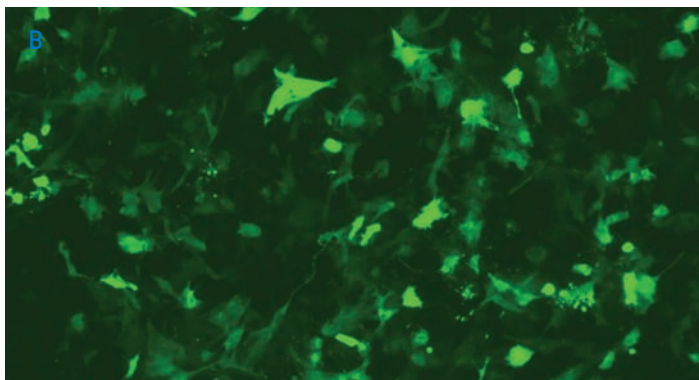
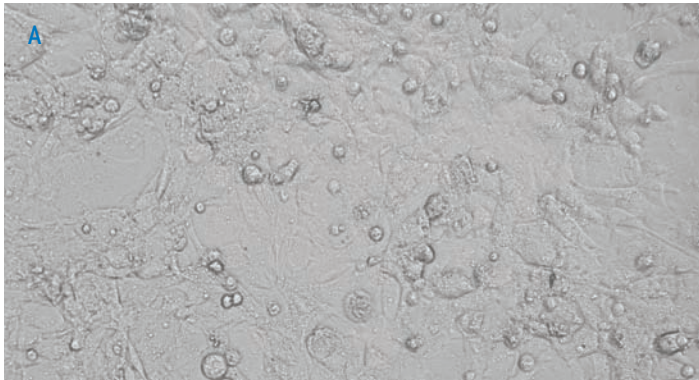


Amaxa™ Nucleofector™ protocol for mouse embryonic fibroblasts (MEF; Lonza)

Example for Nucleofection™ of MEF cells



Average transfection efficiency of mouse embryonic fibroblasts. 2×10^6 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)	2.25 ml
supplement (0.45 ml + 10% overfill)	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 10⁶ 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 10³ cells/cm²
- 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 10⁶ cells
 - 1–5 µg plasmid DNA (in 1–5 µl H₂O 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% CO₂ 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 10⁶ 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% CO₂ 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™ References

www.lonza.com/nucleofection-citations

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Please note that the Amata™ 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 GmbH.

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