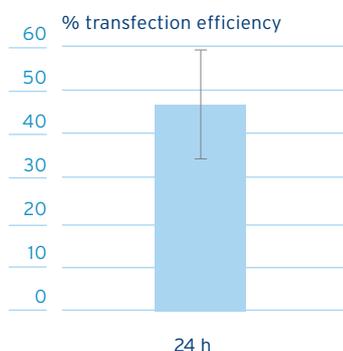




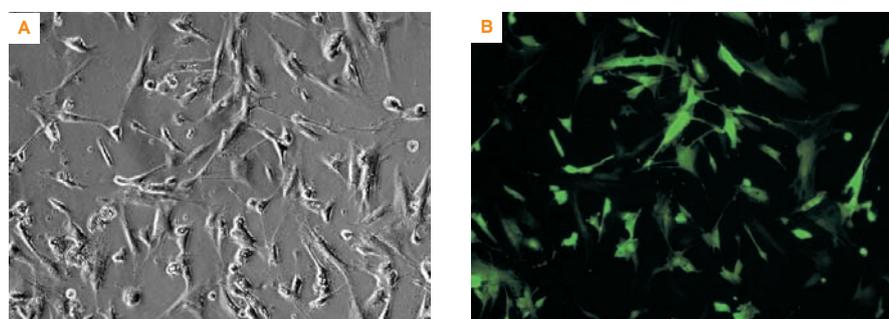
Human MSC Nucleofector® Kit

for Human Mesenchymal Stem Cells

Cell type	Origin	Human Mesenchymal Stem Cells (MSC), cryopreserved [Clonetics/BioWhittaker; Cat. No. PT-2501].
	Morphology	Cells with large nuclei and long spindle like protrusions..



Example for nucleofection® of Human MSC



Transfection efficiencies of Human Mesenchymal Stem cells 24 hours post nucleofection. Cells were nucleofected using program **U-23** and 5 µg of a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^b.

Human MSC were nucleofected using the Human MSC Nucleofector Kit and a plasmid encoding the fluorescent protein eGFP. **24 hours** post-nucleofection cells were analyzed by light (**A**) and fluorescence microscopy (**B**). Transfection efficiencies of around 80% can be reached with eGFP.

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1

Procedure outline & important advice

1.



Procedure outline

Preparation of cells.
(For details see 3.3.)

Important advice

- › Use MSCGM BulletKit (stored < 2 d at 4°C)
- › Passage interval: after reaching 70% confluency.
- › Last passage before nucleofection: 6-7 days.
- › Passage number <9.
- › Confluency before nucleofection: 85%.

2.



Combine the cells of interest, DNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette.
(For details see 3.5.)

Contents of one nucleofection sample:

- › 4-5 x 10⁵ cells (optimal cell number)
- › 2 µg highly purified plasmid DNA (in max. 5 µl).
- › 100 µl Human MSC Nucleofector Solution.

Perform each sample separately to avoid storing the cells longer than 15 min in Human MSC Nucleofector Solution.

3.



Choose the cell-type specific program, insert the cuvette into the Nucleofector and press the start button "X".
(For details see 3.5.)

- › Optimal Nucleofector program: **U-23** (for high transfection efficiency) or **C-17** (for high cell survival).

4.



Rinse the cuvette with culture medium and transfer the cells into the culture dish.
(For details see 3.5.)

- › Remove sample from the cuvette immediately.
- › Use amaxa certified pipette.
- › Transfer directly to 37°C.

2

Product description

Cat. No.	VPE-1001
Kit components	2.25 ml Human MSC Nucleofector Solution 0.5 ml Supplement 10 µg pmaxGFP (0.5 µg/µl in 10 mM Tris pH 8.0) 25 certified cuvettes 25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector Solution and Supplement at 4°C, pmaxGFP™ at -20°C. The expiry date is printed on the Solution Box.

3

Protocol



3.1 › **Required reagents**

Medium

MSCGM - Mesenchymal Stem Cell Growth Medium BulletKit [Clonetics/BioWhittaker; Cat. No. PT-3001]. Note: We recommend storing 40 ml aliquots of prepared medium at -80°C.

Do not use medium stored for more than two days at 4°C because this can lead to an increase in cell mortality after nucleofection.

Trypsin treatment We recommend using ReagentPack [Clonetics/BioWhittaker; Cat. No. CC-5034].



3.2 › **DNA preparation and quality**

The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN® EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit). The purified DNA should be resuspended in deionised water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN® protocol.





3.3 › Cell culture

Culture conditions Replace medium every 2 days
(5-6 ml medium per 75 cm² flask).

Passage interval Cells should be passaged after reaching 70-80% confluency.

Seeding conditions At least 2x10⁵ cells per flask (75 cm²).

Culture conditions before nucleofection

- › The cells should be passaged preferably 6-7 days before nucleofection.
- › Do not use cells after passage number 9 for nucleofection.
- › Cells should be nucleofected after reaching 85% confluency.

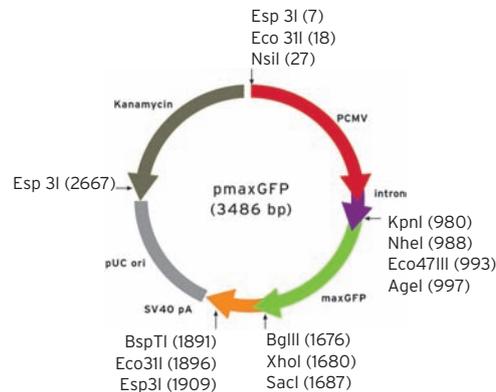
Note

Contamination of cell culture with mycoplasma is a widely spread phenomenon that might negatively influence experimental results. We recommend the use of Primocin™ [Cat. No. VZA-1021], a new antibiotic formula specifically developed to protect sensitive primary cells from mycoplasma infection and microbial contaminations. Add it directly to the cell culture medium without further need of Pen/Strep or other antibiotics. For more information and ordering info see www.amaxa.com/antibiotics.

3.4 › Important controls

1. Positive control

We strongly recommend establishing the Nucleofector technology with the positive control vector **pmaxGFP™** as provided in this kit. pmaxGFP™ encodes the green fluorescent protein (GFP) from copepod *Potellina sp.*. Just like eGFP expressing cells, maxGFP expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



2. Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection or amount/purity of DNA on cell viability.

control 1 Recommended amount of cells in Nucleofector Solution with DNA but without application of the program (alternatively: untreated cells)
(Cells + Solution + DNA - program)

control 2 Recommended amount of cells in Nucleofector Solution without DNA with application of the program **(Cells + Solution - DNA + program)**

3.5 › Nucleofection protocol

Preparation of Nucleofector Solution

Add 0.5 ml Supplement to 2.25 ml Nucleofector Solution and mix gently. The Nucleofector Solution is now ready to use and is stable for 3 months at 4°C.
Note the date of addition on the vial.

One nucleofection sample contains

- › **4-5 x10⁵ cells**
- › **2 µg plasmid DNA (in 1-5 µl H₂O or TE) or 2 µg pmax GFP or 0.5-3 µg siRNA**
- › **100 µl Human MSC Nucleofector Solution**

Minimal cell number: 2x10⁵ cells (a lower cell number may lead to a major increase in cell mortality). Maximum cell number: 6 x 10⁵.

For more details about the nucleofection of siRNA:
www.amaxa.com/RNAi

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **2 µg** DNA for each sample.
3. Pre-warm the supplemented Human MSC Nucleofector Solution to room temperature. Pre-warm an aliquot of culture medium containing serum and supplements at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6-well plates by filling the appropriate number of wells with 1 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO₂ incubator.
5. Remove the medium from the tissue culture. Wash cells once with HBSS [Reagent-Pack], using at least the same volume of HBSS as culture medium. Aspirate and discard the HBSS.

6. Harvest the cells by trypsinization: Add trypsin-EDTA solution [ReagentPack] to cover the cell monolayer, and gently swirl the dish/flask to ensure an even distribution of the solution. Place the dish/flask in an incubator at 37°C until the cells start to detach (usually after 3-5 minutes). Remove the dish/flask from the incubator. Tap against the sides of the dish/flask to improve detachment. Carefully check the process under a microscope to avoid overexposure of cells to trypsin. If necessary, prolong the incubation time for two more minutes at 37°C. Once the majority of cells (>90%) have been dislodged, add TNS [ReagentPack] to inactivate trypsin. Gently resuspend and remove the cells from the flasks by pipetting.
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**4-5x10⁵ cells** per nucleofection sample) at **200xg** for **10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature Human MSC Nucleofector Solution to a final concentration of **4-5x10⁵ cells/100µl**. Avoid storing the cell suspension longer than **15 min** in Human MSC Nucleofector Solution, as this reduces cell viability and gene transfer efficiency.



Nucleofection

Important: Steps 10-14 should be performed for each sample separately.

10. Mix 100 µl of cell suspension with **2 µg** DNA.
11. Transfer the nucleofection sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Insert the cuvette into the cuvette holder and rotate the turning wheel clockwise to the final position. Select the program **U-23** (for high transfection efficiency) or **C-17** (for high cell survival). Press the "X" button to start the program (see Nucleofector Manual for details).
13. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells. Add 500 µl of the pre-warmed culture medium containing serum and supplements and transfer the sample into the prepared 6-well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.
14. Press the "X" button to reset the Nucleofector.
15. Repeat steps 10-14 for the remaining samples.
16. If you have incubated the samples in 1.5 ml microcentrifuge tubes, transfer them into the prepared 6-well plates.
17. Incubate cells in a humidified 37°C/5% CO₂ incubator. After 2 hours of incubation viability of cells can be evaluated by proportion of cells attached to the culture wells.



Cultivation post nucleofection

18. Depending on the gene, expression is often detectable after 6-8 hours. If this is not the case, the incubation period may be prolonged to 24 hours.

4

Recommended literature

Nucleofection references

1. Tuli R, *et al.* J Biol Chem (2003); 278 (42): 41227-41236.

For an up-to-date list of all Nucleofector references, please refer to:
www.amaxa.com/citations

* amaxa's Nucleofector® process, Nucleofector® device and Nucleofector® Solutions are covered by PCT applications PCT/EP01/07348, PCT/DE02/01489, PCT/DE02/01483 and other pending patents and domestic or foreign applications corresponding thereto.

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