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# MEF Nucleofector® Kit 1 and 2

for Mouse Embryonic Fibroblasts (MEF)

**MEF display significant phenotypic variations** which depend on the strain, the genetic background of the they are isolated from as well as the immortalization strategy. With the Test Instructions included in the **MEF Starter Nucleofector Kit** (Cat.No. VPD-1006) you can determine the optimal program and Nucleofector Solution for your MEF line before using one of the MEF Nucleofector Kits.

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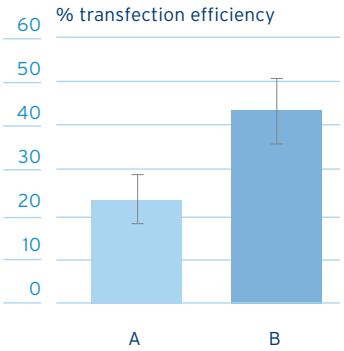
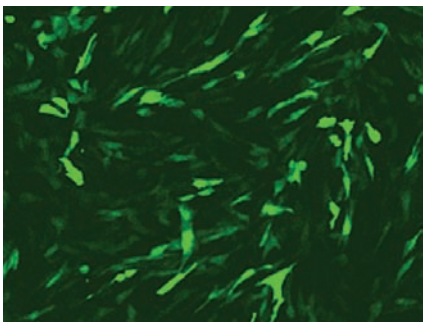
|---|---|---|
| **Cell type** | **Origin** | Primary embryonic fibroblasts, isolated from mouse embryos and immortalized by frequent passaging or SV40 transformation. |
| | **Morphology** | Fibroblastoid. |



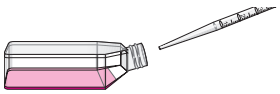
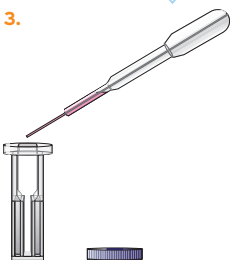
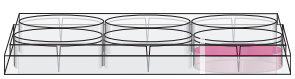

MEF were nucleofected with program **A-23** and a plasmid encoding the enhanced green fluo-rescent protein eGFP. **24 hours** post nucle-ofection, the cells were analyzed by fluorescence microscopy. Cell viability ranges between 60-80%. (A) Mouse strain: C57BL/6, spont-aneously immortalized, passage 20, 1.5 µg eGFP (Courtesy of Dr. H. Hermanns and Prof. P.H. Heinrich, University of Aachen, Germany). (B) Mouse strain: C57B6Jx129SV, SV40-transformed, passage 4, 7 µg eGFP (Courtesy of Prof. Saftig, University of Kiel, Germany).

**Example for nucleofection® of MEF with eGFP cDNA**



Spontaneously immortalized mouse embryonic fibroblasts (strain:C57B6Jx129SV) were nucleofected using the MEF Nucleofector Kit 1, program **A-23** and a plasmid encoding the enhanced green fluorescent protein eGFP. **24 hours** post nucleofection, the cells were analyzed by fluorescence microscopy (Photograph courtesy of Dr. H. Hermanns and Prof. P.H. Heinrich, University of Aachen, Germany).

## 1 Procedure outline & important advice

| | Procedure outline | Important advise |
|---|---|---|
| 1.  | Isolation of cells. (For details see 3.3.) | › For initial experiments please use the **MEF Starter Nucleofector Kit** to determine the optimal program and Nucleofector Solution.<br>› Use DMEM supplemented with 2 mM GlutaMAX, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% FCS.<br>› Confluency before nucleofection: 50-60%<br>› For isolation and culture refer to the literature in chapter 4. |
| 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:**<br>› 2 x $10^6$ cells (optimal cell number)<br>› 3-10 µg highly purified plasmid DNA (in max. 5 µl).<br>› 100 µl MEF Nucleofector Solution 1 or 2.<br><br>Perform each sample separately to avoid storing the cells longer than 20 min in MEF Nucleofector Solution 1 or 2. |
| 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: **A-23** or **T-20**. (program depends on results from experiments with MEF Starter Nucleofector Kit.) |
| 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.<br>› Use amaxa certified pipette.<br>› Transfer directly to 37°C. |

## 2 Product description

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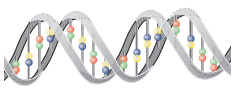
|---|---|
| Cat. No. | **VPD-1004** MEF 1 Nucleofector Kit or<br>**VPD-1005** MEF 2 Nucleofector Kit |
| Kit components | 2.25 ml **MEF 1 or MEF 2 Nucleofector Solution**<br>0.5 ml Supplement 1<br>10 µg pmaxGFP (0.5 µg/µl in 10 mM Tris pH 8.0)<br>25 certified cuvettes<br>25 plastic pipettes |
| Size | 25 reactions |
| Storage and stability | Store all reagents at 4°C.<br>The expiry date is printed on the Solution Box. |

## 3 Protocol

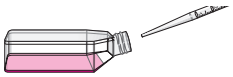
### 3.1 › Required reagents

**Medium** DMEM [Invitrogen/Gibco; Cat. No. 61965-026] supplemented with 2 mM GlutaMAX, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS).

### 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 medium** DMEM [Invitrogen/Gibco; Cat. No. 61965-026] supplemented with 2 mM GlutaMAX, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS).

**Culture conditions before nucleofection**

› Plate 3-4x$10^6$ cells per 150 mm dish to get **50-60% confluence** before nucleofection.

**Note**

**This protocol only gives an outline for the isolation and culture of primary MEF. Please refer to more detailed protocols in the literature before starting the experi ments.**

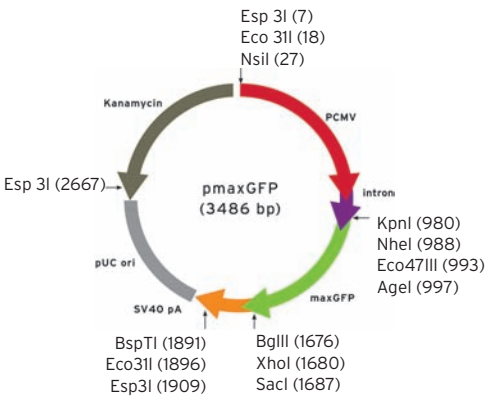
**Note**

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 *Pontellina p.* 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 viabilty.

**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

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|---|---|
| **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** | **› 2 x $10^6$ cells**<br>**› 3-10 µg plasmid DNA (in 1-5 µl $H_2O$ or TE) or 2 µg pmax GFP or 0.5-3 µg siRNA**<br>**› 100 µgl MEF Nucleofector Solution 1 or 2**<br><br>Minimal cell number: 8x$10^5$ cells (a lower cell number may lead to a major increase in cell mortality). Maximum cell number: 5 x $10^6$. |
| | For more details about the nucleofection of siRNA: **www.amaxa.com/RNAi** |

**Preparation of samples**

1. Prepare the required number of cells.
2. Prepare **3 - 10 µg** DNA for each sample.
3. Pre-warm the supplemented MEF Nucleofector Solution 1 or 2 to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µgl per sample).
4. Prepare 100 mm plates, add 8 ml culture medium per sample and pre-incubate plates in a humidified 37°C/5% $CO_2$ incubator.
5. Resuspend the cells in room temperature MEF Nucleofector Solutions 1 or 2 to a final concentration of **2 x $10^6$ cells/100 µl**. Avoid storing the cell suspension longer than **20 min** in MEF Nucleofector Solution 1 or 2, as this reduces cell viability and gene transfer efficiency.

**Important: Steps 6 - 10 should be performed for each sample separately.**

**Nucleofection**

6. Mix 100 µgl of cell suspension with **3 - 10 µg DNA**.
7. 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 cuvette with the blue cap.
8. Insert the cuvette into the cuvette holder and rotate the turning wheel clockwise to the final position. Select programs **A-23** or **T-20** depending on the program with the best results after evaluation with the MEF Starter Nucleofector Kit. Press the "X" key to start the program.
9. **To avoid damage to the cells remove the samples from the cuvette immediately after 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 and transfer the sample into the prepared dishes. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.
10. Press any key to reset the Nucleofector.
11. Repeat steps 6 - 10 for the remaining samples.
12. If you have incubated the samples in 1.5 ml microcentrifuge tubes, transfer them into the prepared dishes.

**Cultivation post nucleofection**

13. Incubate cells in a humidified 37°C/5% $CO_2$ incubator.
14. Depending on the gene, expression is often detectable after 6 - 8 hours. If this is not the case, the incubation period may be prolonged. After 24 - 48 hours of incubation viability of cells can be evaluated by the proportion of cells attached to the dishes.

## 4 Recommended literature

**Nucleofector references**

1. Johnson *et al.* (1995) Nucleic Acid Res. 23: 1273-1275.
2. Hogan *et al.* (1995) »Manipulating the mouse embryo«, Cold Spring Harbor Laboratory Press.

**Additional references**

1. Verrecchia F et al, J Biol Chem. 2003;278(3):1585-93.
2. Verrecchia F et al, EMBO Rep.2002;3(11):1069-1074.

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