MEF Starter Nucleofector® Kit

for Mouse Embryonic Fibroblasts (MEF)

MEF display significant phenotypic variations which depend on the strain, the genetic background of the mice 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.

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

Experimental Setup

Solution	MEF 1		MEF 2	
	Nucleofector I	Nucleofector II	Nucleofector I	Nucleofector II
sample 1	A-23	A-023	-	-
sample 2	T-20	T-020	-	-
sample 3	-	-	A-23	A-023
sample 4	-	-	T-20	T-020

Note

We recommend including one control sample with cells suspended in MEF Nucleofector Solution 1 or 2 together with 5 µg DNA, with no program.

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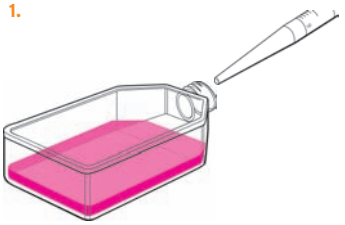
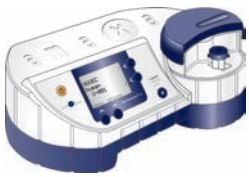
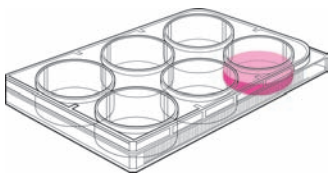
1 Procedure outline & important advice

	Procedure outline	Important advice
1. 	Preparation of cells. (For details see 3.3.)	› Use DMEM supplemented with 2 mM GlutaMAX, 100 U/ml penicillin, 100 µg/ml streptomycin and **10% FCS**. › Confluency before nucleofection: 50-60% › 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:** › 2 x 10^6 cells (optimal cell number) › 5 µg highly purified plasmid DNA (in max. 5 µl). › 100 µl 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.)	› Please follow experimental set-up on page 1.
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.	**VPD-1006**
Kit components	0.45 ml **MEF 1 Nucleofector Solution**
	0.45 ml **MEF 2 Nucleofector Solution**
	0.1 ml Supplement 1
	0.1 ml Supplement 1
	20 µg pmaxGFP™ (0.5 µg/µl in 10 mM Tris pH 8.0)
	10 certified cuvettes
	10 plastic pipettes
Size	10 reactions
Storage and stability	Store Nucleofector Solution, Supplement and maxGFP at 4°C,, For long term storage pmaxGFP is ideally stored at -20°C. The expiry date is printed on the Solution Box.

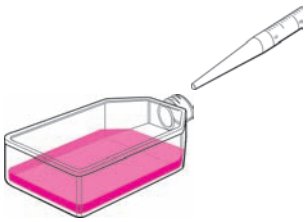
3 Protocol

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 deionized 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

This protocol only gives an outline for the culture of primary MEF. Please refer to more detailed protocols on isolation and cell culture in the literature (chapter 4) before starting the experiments.

Culture conditions before nucleofection

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

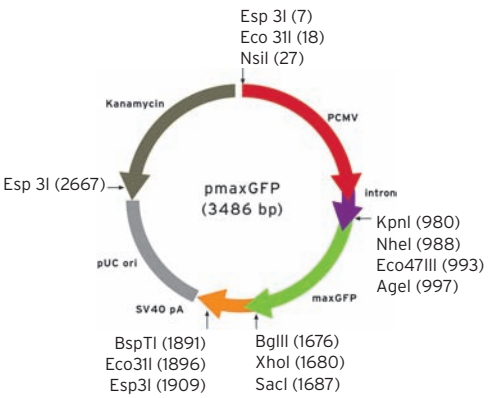
Note

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Primocin™ [Cat. No. VZA-1021], a new antibiotic formulation 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 and vector information

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.



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)**

Vector information

If using IRES sequences in your vectors, please remember that the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some cell types may not be expressed at all. As alternatives we suggest either: co-transfecting two (or more) plasmids, using one plasmid with each gene under the control of its own promoter, or making a GFP fusion.

3.5 › Nucleofection protocol

Preparation of Nucleofector Solution

Add 0.1 ml Supplement to 0.45 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**
- **5 µg plasmid DNA (in 1-5 µl H_2O or TE) or 2 µg pmaxGFP or 0.5-3 µg siRNA**
- **100 µl MEF Nucleofector Solution 1 or 2**

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

Preparation of samples

1. Prepare 10 x 10^6 cells
2. Prepare **5 µg** DNA for each sample.
3. Pre-warm the supplemented MEF Nucleofector Solutions 1 and 2 to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare five 100 mm dishes, 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 and 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 µl of cell suspension with **5 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/A-023** and **T-20/T-020** (following the experimental set-up indicated on page 1). Press the "X" button 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 the "X" button 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.
13. Incubate cells in a humidified 37°C/5% CO_2 incubator.

Cultivation post nucleofection

14. After 24-48 hours of incubation transfection efficiency can be analyzed and viability of cells can be evaluated by proportion of cells attached to the dishes. These results will allow you to determine the best combination of Nucleofector program and Nucleofector Solution for your MEF.

4 Recommended literature

Additional 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