

Optimization Protocol

for Cell Line Optimization Nucleofector® Kit



Application



The Cell Line Optimization Nucleofector Kit enables you to optimize the nucleofection conditions of a cell line of your choice, using a combination of specific Nucleofector programs and Solutions, to transfect a cell line of your choice. To view an up-to-date list of all cell lines for which either an Optimized Protocol or customer data exist, refer to our website at:

www.amaxa.com/celldatabase

Overview

1 Solution	L 	V 
program 1	A - 0 2 0	A - 0 2 0
program 2	T - 0 2 0	T - 0 2 0
program 3	T - 0 3 0	T - 0 3 0
program 4	X - 0 0 1	X - 0 0 1
program 5	X - 0 0 5	X - 0 0 5
program 6	L - 0 2 9	L - 0 2 9
program 7	D - 0 2 3	D - 0 2 3



Step 1 The cell line of interest is transfected with the Nucleofector Solutions L and V in combination with seven different Nucleofector programs.

Step 2 The Nucleofector Solution and program which result in highest transfection efficiencies with lowest mortality are selected.

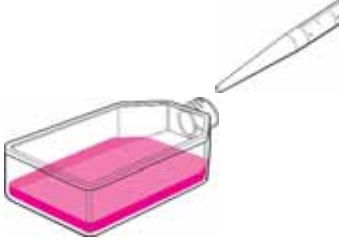
Step 3 A further fine tuning of the nucleofection conditions can be performed with the help of our Scientific Support Team.

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Procedure outline & important advice

1.



Procedure outline

Culturing of cells before nucleofection.
(For details see 7.1 for suspension cells or 8.1 for adherent cells.)

Important advice

Suspension cells

- › Passage 1-2 days before nucleofection.
- › Cells must be in their logarithmic growth phase.

Adherent cells

- › Passage 2-3 days before nucleofection.
- › Cells should be nucleofected at 70-85% confluency.

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 7.2 for suspension cells or 8.2 for adherent cells.)

Contents of one nucleofection sample:

- › 1 x 10⁶ to 5 x 10⁶ cells
- › 2 µg pmaxGFP
- › 100 µl Nucleofector Solution

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

3.

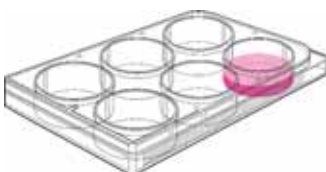


Choose the cell-type specific program. Insert the cuvette into the Nucleofector and press the start button "X".
(For details see 7.2 for suspension cells or 8.2 for adherent cells.)

- › Select the appropriate Nucleofector program as indicated on page 3 (chapter 4).

- › Software requirements
Nucleofector I Version **V2.4** or higher
Nucleofector II Version **S3.5** or higher

4.



Rinse the cuvette with culture medium using an amaxa certified pipette. Transfer the cells into the culture dish. (For details see 7.2 for suspension cells or 8.2 for adherent cells.)

- › Using an amaxa certified pipette, immediately remove sample from the cuvette with 500 µl prewarmed medium.
- › Transfer directly to 37°C.
- › Do not disturb cells for 24h.

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Product description

Cat. No.	VCO-1001N
Kit components	0.9 ml Nucleofector® Solution L 0.9 ml Nucleofector® Solution V 2 x 0.2 ml Supplement 2 x 20 µg pmaxGFP™ (0.5 µg/µl in 10 mM Tris pH 8.0) 18 certified cuvettes 18 plastic pipettes
Size	18 reactions
Storage and stability	Store Nucleofector Solution, Supplement and pmaxGFP at 4°C. For long term storage pmaxGFP is ideally stored at -20°C. The expiry date is printed on the Solution Box.

Optimization guidelines

Step 1

The first set of experiments is comprised of 18 reactions:

Two different Cell Line Nucleofector Solutions L and V are tested in combination with 7 different Nucleofector programs plus 2 controls. The Nucleofector Solution and program with the highest efficiency and lowest mortality are selected. For further transfections, order the respective Cell Line Nucleofector Kit and use it in combination with the selected program.

Step 2 (optional)

To maximize nucleofection efficiency, we recommend establishing a second set of experiments based on the best results obtained. For this purpose submit your complete results to our Scientific Support Team and within one workday we will suggest additional programs to be tested in combination with the best Nucleofector Solution.

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Experimental set-up

Nucleofector Solution L

sample	maxGFP	program
sample 1	+	A - 0 2 0
sample 2	+	T - 0 2 0
sample 3	+	T - 0 3 0
sample 4	+	X - 0 0 1
sample 5	+	X - 0 0 5
sample 6	+	L - 0 2 9
sample 7	+	D - 0 2 3
sample 8	+	-
sample 9	-	T - 0 2 0

Nucleofector Solution V

sample	maxGFP	program
sample 10	+	A - 0 2 0
sample 11	+	T - 0 2 0
sample 12	+	T - 0 3 0
sample 13	+	X - 0 0 1
sample 14	+	X - 0 0 5
sample 15	+	L - 0 2 9
sample 16	+	D - 0 2 3
sample 17	+	-
sample 18	-	T - 0 2 0

5

DNA preparation and quality



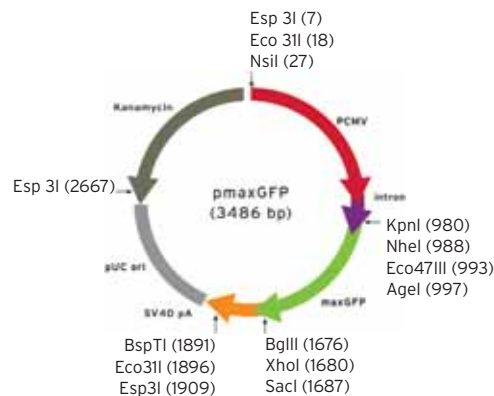
The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN® EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit]. The 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. **The ratio should be at least 1.8 for nucleofection.**

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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 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).**

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.

7

Protocol for suspension cell lines

7.1 › **Cell culture**

For commercially available cell lines we recommend following the instructions of the supplier regarding culture medium and supplements, as well as, passaging and seeding conditions. Best nucleofection results will be obtained with standardized cell culture conditions.

Culture conditions before nucleofection

- › The cells should be passaged 1-2 days before nucleofection.
- › For nucleofection, cells must be in their logarithmic growth phase.

Note

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Normocin™[Cat. No. VZA-1001], a new antibiotic formulation specifically developed to protect sensitive cell lines 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.

7.2 › **Nucleofection protocol**

Preparation of Nucleofector® Solution

Add 0.2 ml Supplement to 0.9 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

- › **1x10⁶ to 5x10⁶ cells**
- › **2µg pmaxGFP**
- › **100 µl Nucleofector Solution**

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



Preparation of samples

1. Cultivate the required number of cells (**1.8x10⁷ to 9x10⁸ cells for 18 experiments**).
2. Pre-warm the supplemented 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, i.e. 9 ml for 18 experiments).
3. Prepare 12-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.
4. Take an aliquot of cell culture and count the cells to determine the cell density.
5. Take the number of cells required for one Nucleofector Solution (**9x10⁶ to 4.5x10⁷ cells for 9 samples of each Nucleofector Solution**). Centrifuge the cells at **90xg at room temperature for 10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
6. Resuspend the pellet in 1 ml Nucleofector Solution to a final concentration of **1x10⁶ to 5x10⁶ cells/100 µl**. Avoid storing the cell suspension longer than **15 min** in Nucleofector Solution, as this reduces cell viability and gene transfer efficiency.
7. Mix 800 µl of cell suspension (8 samples) with **16 µg pmaxGFP**. Keep 100 µl of cell suspension as control without DNA.

Important: Steps 8-11 should be performed for each sample separately.

8. Transfer a 100 µl 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.
9. Select the appropriate Nucleofector program, as indicated in chapter 4 (see Nucleofector I or Nucleofector II Manual for details). Insert the cuvette into the cuvette holder (Nucleofector I: rotate carousel to final position) and press the "X" button to start the program.
10. **To avoid damage to the cells remove the samples from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium to the cuvette and transfer the sample into the prepared 12-well plate. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block. 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.
11. Press the "X" button to reset the Nucleofector.

12. Repeat steps 8-11 for the remaining samples and then continue from step 5 with the next Nucleofector Solution.

13. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 12-well plates.
14. Incubate cells in a humidified 37°C/5% CO₂ incubator. Following nucleofection, gene expression should be analyzed at different times. Depending on the reporter gene, expression is often detectable after 3-8 hours. If this is not the case, the incubation period may be prolonged to 24 hours



Nucleofection



Cultivation post nucleofection

8

Protocol for adherent cell lines

8.1 › **Cell culture**

For commercially available cell lines we recommend following the instructions of the supplier regarding culture medium and supplements, as well as, passaging and seeding conditions. Best nucleofection results will be obtained with standardized cell culture conditions.



For cells grown in high-calcium medium, such as Dulbecco's modified Eagle medium (DMEM), you may use a low calcium medium like RPMI for the transfer from the cuvette into the plate (see 8.2 steps 2 and 12).

Culture conditions before nucleofection

- › The cells should be passaged 2-3 days before nucleofection.
- › Cells should be nucleofected after reaching 70-85% confluency. Higher cell densities may cause lower nucleofection efficiencies.

Note

Contamination of cell culture with mycoplasma is a widely spread phenomenon that might negatively influence experimental results. We recommend the use of Normocin™[Cat. No. VZA-1001], a new antibiotic formula specifically developed to protect sensitive cell lines 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.

8.2 › **Nucleofection protocol**

Preparation of Nucleofector Solution

Add 0.2 ml Supplement to 0.9 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

- › **1x10⁶ to 5x10⁶ cells**
- › **100 µl Nucleofector Solution**
- › **2 µg pmaxGFP**



For more details about the nucleofection of siRNA:

www.amaxa.com/RNAi

Preparation of samples

1. Cultivate the required number of cells (**1.8x10⁷ to 9x10⁷ cells for 18 experiments**).
2. Pre-warm the supplemented Nucleofector Solution to room temperature. Pre-warm an aliquot of culture medium containing serum/supplements (see 8.1) at 37°C in a 50 ml tube (500 µl per sample, i.e. 15 ml for 30 experiments).
3. Prepare 6-well plates by filling the appropriate number of wells with 1.5 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO₂ incubator.
4. Remove the medium from the cultured cells. Wash cells once with PBS. Aspirate and discard PBS.
5. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA (see Nucleofector Manual, for details).
6. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
7. Take the number of cells required for one Nucleofector Solution (**9x10⁶ to 4.5x10⁷ cells for 9 samples of each Nucleofector Solution**). Centrifuge the cells at **90xg at room temperature for 10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
8. Resuspend the pellet in 1 ml Nucleofector Solution to a final concentration of **1x10⁶ to 5x10⁶ cells/100 µl**. Avoid storing the cell suspension longer than 15 min in Nucleofector Solution, as this reduces cell viability and gene transfer efficiency.
9. Mix 800 µl of cell suspension (8 samples) with **16 µg pmaxGFP**. Keep 100 µl of cell suspension as control without DNA.



Nucleofection

- Important: Steps 10-13 should be performed for each sample separately.**
10. Transfer a 100 µl 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.
 11. Select the appropriate Nucleofector program, see Nucleofector I or Nucleofector II Manual for details). Insert the cuvette into the cuvette holder (Nucleofector I: rotate carousel to final position) and press the "X" button to start the program.
 12. **To avoid damage to the cells remove the samples from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium to the cuvette and transfer the sample into the prepared 6-well plate. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block. 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.
 13. Press the "X" button to reset the Nucleofector.
 14. **Repeat steps 10-13 for the remaining samples and then continue from step 8 with the next Nucleofector Solution.**



**Cultivation
post nucleofection**

15. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6-well plates.
16. Incubate cells in a humidified 37°C/5% CO₂ incubator. Following transfection, gene expression should be analyzed at different times. Depending on the reporter gene, expression is often detectable after 3-8 hours. If this is not the case, the incubation period may be prolonged to 24 hours.

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