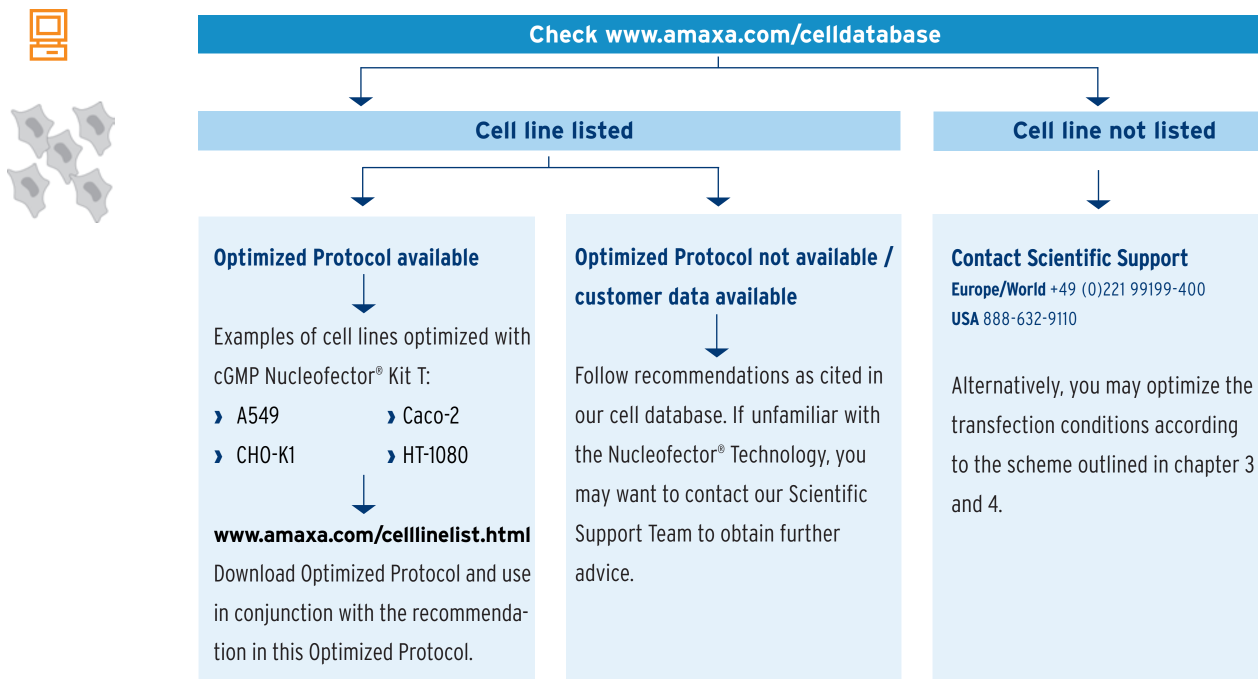
Optimization Protocol

for **cGMP Nucleofector® Solution T [Lot No. 00219-060815]**
for **cGMP Nucleofector® Supplement 1 [Lot No. 00221-060818]**

amaxa has developed Optimized Protocols for many cell lines containing important and valuable information. Please follow the scheme below to determine the best way of establishing the Nucleofector® Technology with your cell line.



Note amaxa is continously developing Optimized Protocols for more cell lines. To be kept up-to-date please check **www.amaxa.com/celldatabase**.

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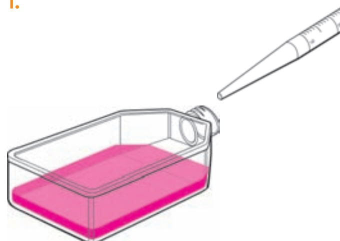
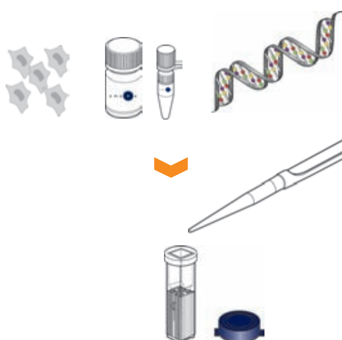
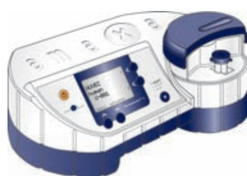
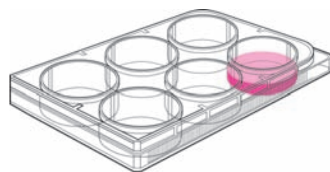
1 Procedure outline & important advice

	Procedure outline	Important advice
1. 	Culturing of cells before nucleofection®. (For details see 7.1 for suspension cells and 8.1 for adherent cells.) Prewarm culture medium to 37°C.	**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 or siRNA and the appropriate cell-type specific cGMP Nucleofector® Solution and transfer to an amaxa certified cuvette. (For details see 7.2 for suspension cells and 8.2 for adherent cells.)	**Contents of one nucleofection® sample:** › Optimal cell number: 1 x 10^6 to 5 x 10^6 › Plasmid DNA: 1 - 5 µg highly purified plasmid DNA › siRNA: start with **30** and **300 nM** › Nucleofector® Solution: 100 µl cGMP Nucleofector® Solution T Avoid leaving the cells in Nucleofector® Solution for extended periods of time (longer than 15 min).
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 and 8.2 for adherent cells.)	› Select the appropriate Nucleofector® program in Optimized Protocol or as per optimization guidelines indicated on page 3.
4. 	Using an amaxa certified pipette, carefully add medium and then remove cell suspension from cuvette. Transfer the cells into the culture dish. (For details see 7.2 for suspension cells and 8.2 for adherent cells.)	› Using an amaxa certified pipette, immediately remove sample from the cuvette with 500 µl prewarmed medium. › Avoid repeated pipetting. › Transfer directly to 37°C.

amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110
scientific-support.US@amaxa.com

2 Product description

Cat. No.	**VGA-1002**
Kit components	2.25 ml **cGMP Nucleofector® Solution T [Lot No. 00219-060815]**
	0.7 ml cGMP Supplement 1 [Lot No. 00221-060818]
	25 certified cuvettes
	25 plastic pipettes
Size	25 reactions
Storage and stability	Store cGMP Nucleofector® Solution and Supplement at 4°C. The expiry date is printed on the Solution Box.

Product identification / certification

cGMP Nucleofector® Solution T and cGMP Nucleofector® Supplement 1 are identified by the respective Lot number printed on the Solution and Supplement vial. Each Lot of cGMP Nucleofector® Solution and Supplement is quality tested and certified. The respective certification of analysis is included in each cGMP Nucleofector® Kit.

3 Optimization guidelines for cell lines not listed in the celldatabase

Note We recommend contacting Scientific Support to discuss the best way of establishing the Nucleofector® Technology with your cell line.

Step 1 The first set of experiments is comprised of 9 reactions: Cell Line Nucleofector® Solutions T is tested in combination with 7 different Nucleofector® programs plus 2 controls.

Step 2 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.

4 Experimental set-up

cGMP Nucleofector® Solution T

sample	Control DNA	Program		
		Nucleofector® I		Nucleofector® II
Sample 1	+	A-20	or	A-020
Sample 2	+	T-20	or	T-020
Sample 3	+	T-30	or	T-030
Sample 4	+	X-01	or	X-001
Sample 5	+	X-05	or	X-005
Sample 6	+	L-29	or	L-029
Sample 7	+	D-23	or	D-023
Sample 8	+	-	or	-
Sample 9	-	T-20	or	T-020

5 Important control and vector information

Important 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.

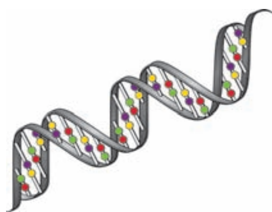
Control 1 Recommended amount of cells in cGMP Nucleofector® Solution with DNA but without application of the program (alternatively: untreated cells) **(Cells + Solution + DNA - program)**

Control 2 Recommended amount of cells in cGMP 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.

6 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® manual.

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 2 - 3 days before nucleofection®.
- For nucleofection®, cells must be in their logarithmic growth phase.

7.2 › Nucleofection® protocol

Preparation of Nucleofector® Solution

Pipet 0.5 ml cGMP Supplement to 2.25 ml cGMP Nucleofector® Solution and mix gently. Please note: To avoid pipetting errors the cGMP Supplement vial contains 0.7 ml cGMP Supplement. After addition of cGMP Supplement to cGMP Nucleofector® Solution 0.2 ml cGMP Supplement will remain in the vial.
The cGMP 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

- **Optimal cell number: 1 x 10^6 to 5 x 10^6 cells**
- **Plasmid DNA: 1 - 5 µg plasmid DNA (in 1 - 5 µl H_2O or TE)**
- **siRNA: 30 and 300 nM (start range)**
- **Nucleofector® Solution: 100 µl cGMP Nucleofector® Solution T**

For an initial experiment we recommend using 30 and 300 nM siRNA as a minimum. Depending on target and cell type, the minimum effective siRNA concentration may range between 1 nM and 1 µM. For optimal knockdown we also propose performing a timecourse experiment (mRNA: 12 - 72 hours, protein/phenotype: 24 - 96 hours).
For more details about the nucleofection® of siRNA: **www.amaxa.com/RNAi**

amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110
scientific-support.US@amaxa.com

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **1 - 5 µg** DNA for each sample. For siRNA we recommend to start using **30 nM** and **300nM** for each sample.

3. **Pre-warm the supplemented cGMP Nucleofector® Solution T recommended by amaxa 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 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% CO_2 incubator.**
5. Take an aliquot of cell culture and count the cells to determine the cell density.
6. Centrifuge the required number of cells (**1 x 10^6 - 5 x 10^6 cells** per nucleofection® sample) at **90xg** at **room temperature** for **10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
7. Resuspend the pellet in room temperature Nucleofector® Solution T to a final concentration of **1 x 10^6 - 5 x 10^6** cells/100 µl. Avoid storing the cell suspension longer than **15 - 20 min** in cGMP Nucleofector® Solution T, as this reduces cell viability and gene transfer efficiency.

Important: Steps 8-12 should be performed for each sample separately.

Nucleofection®

8. Mix 100 µl of cell suspension with **1 - 5 µg DNA** or the appropriate amount of siRNA.
9. Transfer the 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.
10. Select the appropriate Nucleofector® program (see Nucleofector® Manual for details). Insert the cuvette into the cuvette holder (Nucleofector® I: rotate the turning wheel clockwise to the final position). Press the "X" button to start the program.
11. When the display shows »OK« (nucleofection® process is completed) take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium to the cuvette immediately and transfer the sample into the prepared 12-well plates. 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.

Note: Avoid leaving the cells in cGMP Nucleofector® Solution for extended periods of time (longer than 15 - 20 minutes), as this may reduce cell viability.

12. Press the "X" button to reset the Nucleofector®.
13. Repeat steps 8 - 12 for the remaining samples.
14. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 12-well plates.

Cultivation post nucleofection®

15. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following nucleofection®, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 3 - 8 hours. If this is not the case, the incubation period may be prolonged to 24 hours.

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 3 and 13).

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.

8.2 › Nucleofection® protocol

Preparation of Nucleofector® Solution

Pipet **0.5 ml** cGMP Supplement to 2.25 ml cGMP Nucleofector® Solution and mix gently. Please note: To avoid pipetting errors the cGMP Supplement vial contains 0.7 ml cGMP Supplement. After addition of cGMP Supplement to cGMP Nucleofector® Solution 0.2 ml cGMP Supplement will remain in the vial.
The cGMP 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

- **Optimal cell number: 1 x 10^6 to 5 x 10^6 cells**
- **Plasmid DNA: 1 - 5 µg plasmid DNA (in 1 - 5 µl H_2O or TE)**
- **siRNA: 30 and 300 nM (start range)**
- **Nucleofector® Solution: 100 µl cGMP Nucleofector® Solution T**

For an initial experiment we recommend using 30 and 300 nM siRNA as a minimum. Depending on target and cell type, the minimum effective siRNA concentration may range between 1 nM and 1 µM. For optimal knockdown we also propose performing a timecourse experiment (mRNA: 12 - 72 hours, protein/phenotype: 24 - 96 hours).
For more details about the nucleofection® of siRNA:
www.amaxa.com/RNAi

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **1 - 5 µg DNA** for each sample. For siRNA we recommend to start using **30 nM** and **300nM** for each sample.

3. **Pre-warm the supplemented cGMP Nucleofector® Solution T recommended by amaxa to room temperature. Pre-warm an aliquot of culture medium containing serum/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 1.5 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO_2 incubator.**
5. Remove the medium from the cultured cells. Wash cells once with PBS. Aspirate and discard PBS.
6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with culture medium containing serum and supplements (see Nucleofector® Manual for details).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**1×10^6 - 5×10^6** cells per nucleofection® sample) at **90xg** at **room temperature** for **10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature cGMP Nucleofector® Solution T recommended by amaxa to a final concentration of **1×10^6 - 5×10^6** cells/100 µl. Avoid storing the cell suspension longer than **15 - 20 min** in cGMP Nucleofector® Solution T, as this reduces cell viability and gene transfer efficiency.

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

Nucleofection®

10. Mix 100 µl of cell suspension with **1 - 5 µg DNA** or the appropriate amount of siRNA.
11. Transfer the 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. Select the appropriate Nucleofector® program (see Nucleofector® Manual for details). Insert the cuvette into the cuvette holder (Nucleofector® I: rotate the turning wheel clockwise to the final position). Press the "X" button to start the program.
13. When the display shows »OK« (nucleofection® process is completed) take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium to the cuvette immediately and transfer the sample into the prepared 6-well plates. 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.

Note: Avoid leaving the cells in cGMP Nucleofector® Solution for extended periods of time (longer than 15 - 20 minutes), as this may reduce cell viability.

14. Press the "X" button to reset the Nucleofector®.
15. If you have incubated the samples in 1.5 ml microcentrifuge tubes, transfer all samples into the prepared 6-well plates.

Cultivation post nucleofection®

16. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following transfection, gene expression should be analyzed at different times. Depending on the 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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amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110
scientific-support.US@amaxa.com