

## cGMP general Protocol

for Nucleofection of adherent cells

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Please check our cell database:

[www.amaxa.com/celldatabase](http://www.amaxa.com/celldatabase)

to see if an Optimized Protocol or any customer data exists for your specific celltype.

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**Procedure outline & important advice**

1.



**Procedure outline**

Culturing of cells before nucleofection.  
(For details see 2.1.)

**Important advice**

- › For culturing, follow instructions of the cell line supplier.
- › The cells should be passaged 2-3 days before nucleofection.
- › Cells should be nucleofected at 70-80% 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 2.4.)

**Contents of one nucleofection sample:**

- ›  $1 \times 10^6$ - $5 \times 10^6$  cells (optimal cell number).
- › 2 µg highly purified plasmid DNA (in max 5 µl) or 0.5-3 µg siRNA.
- › 100 µl room temperature cGMP Nucleofector Solution.

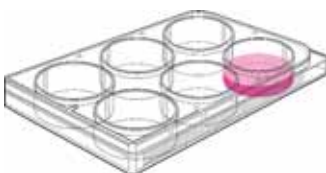
3.



Insert the cuvette into the Nucleofector and choose the cell-type specific program. Press the start button "X".  
(For details see 2.4.)

- › Please refer to the Optimized Protocols for cGMP Nucleofector Kit R, Tor V.

4.

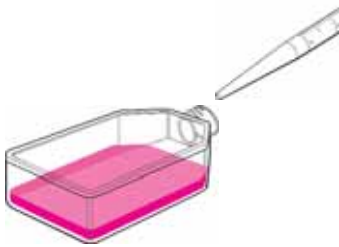


Rinse the cuvette with culture medium using an amaxa certified pipette transfer the cells into the culture dish.  
(For details see 2.4.)

- › Using an amaxa certified pipette, immediately remove sample from the cuvette with 500 µl prewarmed medium.
- › Transfer directly to 37°C.

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**Protocol**



**2.1 › 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 2.4, 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.

**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 cell lines from mycoplasma infection and microbial contaminations. Add it directly to the cell culture medium. For more information and ordering info see: [www.amaxa.com/antibiotics](http://www.amaxa.com/antibiotics)



**2.2 › 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® Manual.

**2.3** › **Important controls**

**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 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)**

**2.4** › **Nucleofection protocol**

**Preparation of Nucleofector Solution**

Add **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**

- › **1x10<sup>6</sup>-5x10<sup>6</sup> cells**
- › **1-5 µg plasmid DNA (in 1-5 µl H<sub>2</sub>O or TE) or 0.5-3 µg siRNA**
- › **100 µl room temperature cGMP Nucleofector Solution**



For more details about the nucleofection of siRNA:  
[www.amaxa.com/RNAi](http://www.amaxa.com/RNAi)

**Preparation of samples**

1. Cultivate the required number of cells.
2. Prepare **1-5 µg DNA or 0.5-3 µg siRNA** for each sample.
3. Pre-warm the supplemented cGMP Nucleofector Solution recommended by amaxa to room temperature. Pre-warm an aliquot of culture medium containing serum/ supplements (see section 2.1) 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 (please see 2.1) and pre-incubate plates in a humidified 37°C/5% CO<sub>2</sub> 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 (**1x10<sup>6</sup>-5x10<sup>6</sup>** 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 recommended by amaxa to a final concentration of **1x10<sup>6</sup>-5x10<sup>6</sup>** cells/100 µl. Avoid storing the cell suspension longer than 15-20 min in cGMP 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 **1-5 µg DNA or 0.5-3 µg 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. Insert the cuvette into the cuvette holder and rotate the turning wheel clockwise to the final position. Select the appropriate Nucleofector program (see Nucleofector Manual for details). Press the "X" key to start the program.
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. Add 500 µl of the pre-warmed culture medium containing serum and supplements (please see 2.1) 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. 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.
14. Press any key 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.
16. Incubate cells in a humidified 37°C/5% CO<sub>2</sub> 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.

**Cultivation  
post nucleofection**

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## Recommended literature

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