BioResearch

Lonza

Amaxa™ cGMP Nucleofector™ Kit V for Adherent and Suspension Cell Lines

Note
Lonza 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.

www.lonza.com/cell-database

Cell line listed	Cell line not listed
Optimized Protocol available Examples of cell lines optimized with cGMP Nucleofector™ Kit V: – HEK293 – COS-1 / COS-7 – MCF-7 – SH-SY5Y **www.lonzabio.com/protocols** Download Optimized Protocol and use in conjunction with the recommendation in this Optimized Protocol.	Alternatively, you may optimize the transfection conditions by using the Amaxa™ Cell Line Optimization Nucleofector™ Kit (VCO-1001). See overview of optimization scheme on the next page.

or

Optimized Protocol not available / customer data available

Follow recommendations as cited in our cell database. If unfamiliar with the Nucleofector™ Technology, you may want to contact our Scientific Support Team to obtain further advice.

Product Description

Cat. No.	VGA-1003
Size	25 reactions
cGMP Nucleofector™ Solution V	2.25 ml
cGMP Supplement 1	0.7 ml
Certified cuvettes	25
Certified plastic pipettes	25

Storage and Stability

Store Nucleofector™ Solution and Supplement at 4°C. The expiration date is printed on the solution box. Once the Nucleofector™ Supplement is added to the Nucleofector™ Solution, it is stable for three months at 4°C.

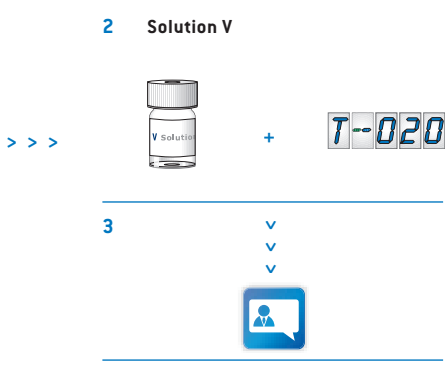
Note
cGMP Nucleofector™ Solutions can only be used with the Nucleofector™ I/II Device. They are not compatible with the 4D-Nucleofector™ or the 96-well Shuttle™.

Product Identification/Certification

cGMP Nucleofector™ Solution V 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.

Overview Optimization Strategy

1 Solution	L	V
Program 1	A-020	A-020
Program 2	T-020	T-020
Program 3	T-030	T-030
Program 4	X-001	X-001
Program 5	X-005	X-005
Program 6	L-029	L-029
Program 7	D-023	D-023



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.

Required Material

– Pipet **0.5 ml** cGMP Supplement to 2.25 ml cGMP Nucleofector™ Solution and mix gently.

Note

To avoid pipetting errors the cGMP Supplement vial contains 0.7 ml cGMP Supplement. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofector™ Solution plus 18 µl of supplement to make 100 µl of total reaction volume.

– Software requirements: version V2.3 or higher for Nucleofector™ I Device; version S3-4 or higher for Nucleofector™ II Device
– Supplemented Nucleofector™ Solution at room temperature
– Supplied certified cuvettes
– Supplied certified plastic pipettes
– Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
– 6-well (for adherent cells) or 12-well (for suspension cells) culture dishes or culture system of your choice
– **For detaching adherent cells**: For commercially available cell lines use e.g. 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA (if not recommended differently by cell supplier)
– **Culture medium**: For commercially available cell lines we recommend following the instructions of the supplier regarding culture medium and supplements
– **Recovery medium (optional for adherent cells)**: 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 culture plate (see chapter 2, note after step 2.11)
– Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample for adherent cells; 1 ml per sample for suspension cells)
– Appropriate number of cells (1–5 x 10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Cell culture Recommendations for Suspension Cells

1.1 For commercially available cell lines we recommend following the instructions of the supplier regarding passaging and seeding conditions. Best Nucleofection™ Results will be obtained with standardized cell culture conditions
1.2 Subculture 1–2 days before Nucleofection™
1.3 Optimal density for Nucleofection™: Cells must be in their logarithmic growth phase

Cell Culture Recommendations for Adherent Cells

1.4 For commercially available cell lines we recommend following the instructions of the supplier regarding passaging and seeding conditions. Best Nucleofection™ Results will be obtained with standardized cell culture conditions
1.5 Subculture 2–3 days before Nucleofection™
1.6 Optimal confluency for Nucleofection™: 70–85%. Higher cell densities may cause lower Nucleofection™ efficiencies

Trypsinization (for Adherent Cells Only)

1.7 For commercially available cell lines we recommend following the instructions of the supplier regarding detaching of cells. You may e.g. use 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA

2. Nucleofection™

One Nucleofection™ Sample Contains

- 1–5 x 10^6 cells (adherent or suspension cells)
- 1–5 µg plasmid DNA
- 100 µl Nucleofector™ Solution

2.1 Prepare culture plates by filling appropriate number of wells with supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator:
Adherent cells: 6-well plates filled with 1.5 ml medium
Suspension cells: 12-well plates filled with 1 ml medium

2.2 **For adherent cells**: Harvest the cells by trypsinization (please see 1.7)

2.3 Count an aliquot of the cells and determine cell density

2.4 Centrifuge the required number of cells (1–5 x 10^6 cells per Nucleofection™ Sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely

2.5 Resuspend the pellet in room temperature Nucleofector™ Solution to a final concentration of 1 x 10^6–5 x 10^6 cells/100 µl

Note
Avoid leaving the cells in Nucleofector™ Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability and gene transfer efficiency

2.6 Mix 100 µl of cell suspension with 1–5 µg DNA or the appropriate amount of siRNA. For siRNA we recommend to start using 30 nM and 300 nM for each sample

2.7 Transfer 100 µl of each aliquot into certified cuvettes. Make sure that the sample covers the bottom of the cuvette and avoid air bubbles while pipetting. Close the cuvette with the cap

2.8 Select the appropriate Nucleofector™ Programs (see Nucleofector™ Manual for details)

2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program by pressing the X-button

2.10 Take the cuvette out of the holder once the program is finished

2.11 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6-well plates (for adherent cells; final volume 2 ml media per well) or 12-well plates (for suspension cells; final volume 1.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

Note:
If very high mortality is observed with adherent cells, a recovery step can be a useful option. Immediately after Nucleofection™, add ~500 µl pre-equilibrated low-calcium media such as RPMI and gently transfer it to the reaction tube. Place the cell suspension in an incubator for 5–10 minutes ("Recovery Step"). Then transfer the sample to the prepared culture dish with culture medium (resulting in a mixed medium until next medium renewal).

3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours. A usual analysis time is 24 hours post Nucleofection™

Additional Information

Up–To–Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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