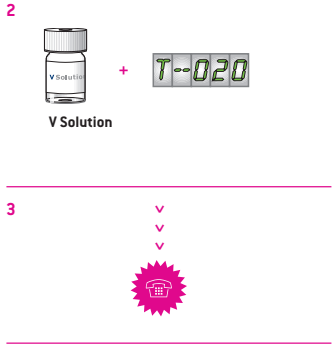
Amaxa® Cell Line Optimization Nucleofector® Kit

For plasmid DNA and/or siRNA

Note 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.lonzabio.com/celldatabase

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.

Product Description

Cat. No.	VCO-1001
Size (reactions)	20
Cell Line Nucleofector® Solution L	0.9 ml
Cell Line Nucleofector® Solution V	0.9 ml
Supplement	2 x 0.2 ml
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	2 x 20 µg
Certified cuvettes	20
Plastic pipettes	20
Storage and stability	Store Nucleofector® Solution, Supplement and pmaxGFP® Vector at 4°C. For long-term storage, pmaxGFP® Vector is ideally stored at -20°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.

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 as optimal condition for this cell line. For further transfections of this cell line, order the respective Cell Line Nucleofector® Kit and use it in combination with the selected program.

– **Step 2** (optional): To maximize Nucleofection® Efficiency or viability post Nucleofection®, 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. On our website (www.lonzabio.com) we provide a form you might use to enter the results achieved with the Cell Line Optimization Kit

Experimental setup

sample	maxGFP®	program*
sample 1	+	A-020
sample 2	+	T-020
sample 3	+	T-030
sample 4	+	X-001
sample 5	+	X-005
sample 6	+	L-029
sample 7	+	D-023
sample 8	+	-
sample 9	-	T-020

sample	maxGFP®	program*
sample 10	+	A-020
sample 11	+	T-020
sample 12	+	T-030
sample 13	+	X-001
sample 14	+	X-005
sample 15	+	L-029
sample 16	+	D-023
sample 17	+	-
sample 18	-	T-020

Nucleofector® Solution L **Nucleofector® Solution**

*Equivalent to A-20, T-20, T-30, X-01, X-05, L-29 and D-23 for Nucleofector® I Device

Required Material

Note **Please make sure that the entire supplement is added to the Nucleofector® Solution. 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.**

– Nucleofector® Device; 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 plastic pipettes
– Supplied pmaxGFP® Vector
– 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, step 2.13)
– Prewarm appropriate volume of culture medium to 37°C (2 ml per sample for adherent cells; 1.5 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 trypsin/EDTA 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)
2 µg pmaxGFP® Vector
100 µl Cell Line Nucleofector® Solution L or V

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 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
2.3 For adherent cells: Harvest the cells by trypsinization (please see 1.7)
2.4 Count an aliquot of the cells and determine cell density
2.5 Prepare **two** aliquots of cell suspension, each with the number of cells required for 9 samples (one aliquot for each Nucleofector® Solution, see optimization guidelines): 9 x 10^6 – 4.5 x 10^7 cells
2.6 Centrifuge the required number of cells **at 90xg for 10 minutes** at room temperature. Remove supernatant completely
2.7 Resuspend each cell pellet carefully in 900 µl room-temperature Nucleofector® Solution

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.**

2.8 Mix 800 µl of each cell suspension (8 samples) with 16 µg pmaxGFP® Vector. Keep 100 µl of cel suspension without DNA
2.9 Transfer 100 µl of each aliquot into certified cuvettes according to the experimental setup (see optimization guidelines; sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.10 **Select the appropriate Nucleofector® Programs according to the experimental setup**
2.11 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.12 Take the cuvette out of the holder once the program is finished
2.13 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 1.5 ml media per well**) or 12-well plates (for suspension cells; **final volume 2 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

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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