Amaxa® Cell Line Nucleofector® Kit V

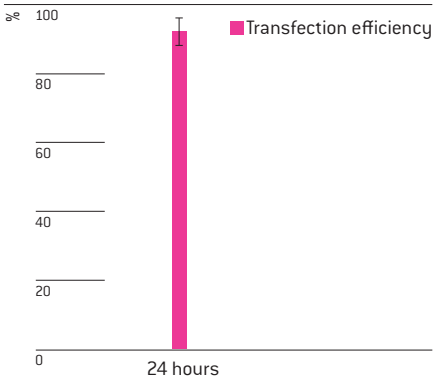
For suspension CHO clones

Chinese hamster ovary; suspension cells
In the table below, Nucleofection® Conditions for a selection of different suspension CHO clones are given. For these clones we recommend to follow the specific culturing recommendations of the respective supplier.

Nucleofection® Conditions successfully tested for selection of various suspension CHO cell clones.

Suspension CHO clone	**CHOK1SV**	**CHO**	**CHO-DG44**	**CHO-DG44 DHFR-**	**CHO-DXB11**	**CHO-S Freestyle**
Cell supplier	Lonza	ECACC		Invitrogen		Invitrogen
Solution	V	V	V	V	V	V
Program	U-023	U-024	U-023	U-030	U-024	U-024
Vector	pmaxGFP®	pmaxGFP®	pmaxGFP®	pmaxGFP®	pmaxGFP®	pmaxGFP®
Cell number	2 million	1 million	1 million	1 million	1.5 million	2 million
Transfection efficiency	80%	92%	80%	93%	69%	80%
Viability	n.a.	82%	80%	95%	95%	92%

Nucleofection® conditions successfully tested for selection of various suspension CHO cell clones. For Nucleofection® of further suspension CHO clones please check **www.lonza.com/cell-database** or contact the Lonza Scientific Support Team.



Average transfection efficiency of suspension CHO cells (ECACC). Suspension CHO cells (ECACC 102307) were transfected with program U-024 and 2.5 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell viability (% PI negative cells) is around 82% 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1003**
Size (reactions)	25
Cell Line Nucleofector® Solution V	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
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.

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
- 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
- 24-well suspension culture dish or culture system of your choice
- **Culture medium**: Follow the specific culturing recommendations of the respective supplier; for the ECACC clone use CHO protein-free medium [SIGMA; Cat. No. C-5467] with 2 mM L-Glutamine
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells: Minimal recommended cell number is $1x10^6$ cells per Nucleofection® sample (a lower cell number leads to increased cell mortality); maximal cell number: 1×10^7 (e.g. for transient protein production)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Do not use cells after passage 30 for Nucleofection®
1.2 Maintain cultures between 3 – 9 x 10^5 cells/ml
1.3 Seed out 2 x 10^5 cells/ml
1.4 Subculture 2 – 3 days before Nucleofection®. Cells should be grown to a density of 5 – 10 x 10^5 cells/ml before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

1 x 10^6 cells (minimal recommended cell number)
1 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution V

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 24-well suspension culture plates by filling appropriate number of wells with 1 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Count an aliquot of the cells and determine cell density
2.4 Centrifuge the required number of cells (1 x 10^6 cells per sample) at 100xg for 8 minutes at room temperature. Remove supernatant completely

2.5 Resuspend the cell pellet carefully in 100 µl room-temperature Nucleofector® Solution per sample

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 Combine 100 µl of cell suspension with 1 – 5 µg DNA, 2 µg pmaxGFP® Vector or 30 nM – 300 nM siRNA (3 – 30 pmol/sample) or other substrates
2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.8 Select the appropriate Nucleofector® Program (for selection of best programs for other CHO clones please check table on first page, go to **www.lonza.com/celldatabase** or contact the Scientific Support Team)
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 24-well plate (up to 1 x 10^6 cells/ml, final volume 1.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

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
3.2 Cells have been successfully tested for transient protein production up to 5 days

Scaling up suspension CHO cell cultures for transient protein production

You can scale up suspension CHO cell culture by pooling several Nucleofection® Samples. You may cultivate cells in Erlenmeyer, spinner or suspension culture flasks. Note that the appropriate shaker or spinner speed and seeding density should be determined and optimized for each culture system. For more protocol details please refer to the Technical Reference Guideline "Transient Protein Production using Nucleofector® Technology" at **www.lonza.com/nucleofection-techlib**

Nucleofection® of suspension CHO cell cultures for generation of stable clones

For more detailed protocol informations please refer to the "Guideline for Generation of Stable Cell Lines" at **www.lonza.com/nucleofection-techlib**

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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References

1. Lattenmeyer, C. *et al.* [2007]. Protein-free transfection of CHO host cells with an IgG-fusion protein: Selection and characterization of stable high producers and comparison to conventionally transfected clones. Biotechnol. Bioeng. 96: 1118-1126.

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