BioResearch

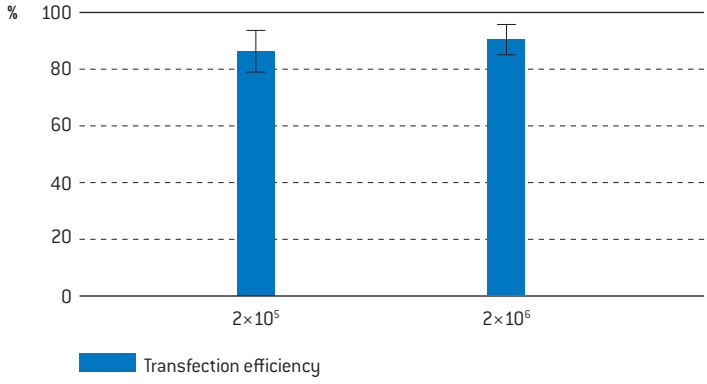
Lonza

Amaxa™ 96-well Shuttle™ Protocol for CHO-S (Invitrogen)

Cell Description

Chinese hamster (Cricetulus griseus) ovary; fibroblastoid cells; (SFM adapted, Invitrogen; cryopreserved).

Example for Nucleofection™ of CHO-S Cells



Transfection efficiency of CHO-S cells 24 hours post Nucleofection™. 2×10^5 or 2×10^6 CHO-S cells were transfected with program 96-FF-137 and 0.4 µg of pmaxGFP™ Vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability is usually around 50 % for both cell numbers after 24 hours.

Product Description

Recommended Kits

SG Cell Line 96-well Nucleofector™ Kit

Cat. No.	V4SC-3096
Size (reactions)	1×96
SG Cell Line 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SC-3960
Size (reactions)	10×96
SG Cell Line 96-well Nucleofector™ Solution	22.5 ml
Supplement	5.0 ml
pmaxGFP™ Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	10

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.

Note

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Required Material

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

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate(s)
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note
Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- Nucleocuvette™ compatible tips: epT.I.P.S. (US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266), Matrix TallTips™ (Matrix Technologies Corp., Cat.No. 7281) or LTS Tips (Rainin Instrument, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S). Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- 96-well culture plates or culture plates of your choice
- Culture medium: CD-CHO Medium (Invitrogen, Cat.No. 10743-011) with 10 ml/l HT-Supplement (Invitrogen, Cat. No. 11067-030) and 8 mM L-Glutamine (Invitrogen, Cat. No. 25030)
- Prewarm appropriate volume of culture medium to 37°C (255 µl per sample)
- Appropriate number of cells (2×10^5 or up to 2×10^6 cells per sample, e.g. for high yield transient protein expression)

1. Pre Nucleofection™

Cell Culture Recommendations

1.1 Replace media every 2–3 days. Do not use cells after passage 25 for Nucleofection™. For more details please refer to supplier's informations
1.2 Passage cells at a maximum density of 2×10^6 to 3×10^6 cells/ml
1.3 Seed out $1–2\times10^5$ cells/ml. For more details please refer to supplier's informations
1.4 Subculture 2 days before Nucleofection™. Cells should be grown to a density of $1–2\times10^6$ cells/ml before Nucleofection™

2. Nucleofection™

One Nucleofection™ Sample Contains

- $2\times10^5–2\times10^6$ cells
- 0.4–1.5 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl SG Cell Line 96-well Nucleofector™ Solution

Note
Since the concentration of plasmid DNA has a strong effect on transient protein yields, it is recommended to test varying amounts of plasmid DNA between 0.4 and 1.5 µg for optimization of protein expression rates.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see device and software manuals)
2.3 Select the appropriate 96-well Nucleofector™ Program **96-FF-137**
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 175 µl* (see note at the end of this chapter) for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator
2.5 Pre-warm an aliquot of culture medium to 37°C (80 µl per sample*)
2.6 Prepare 0.4–1.5 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
2.7 Count an aliquot of the cells and determine cell density
2.8 Centrifuge the required number of cells (2×10^5 or up to 2×10^6 cells per sample) at 90×g for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

2.10 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.11 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.12 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.13 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.14 Resuspend cells with 80 µl* (recommendation for 96-well plates) or desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times.

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 25 µl of resuspended cells to 175 µl pre-warmed medium prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases. However, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

3.1 Recommendation for "pooled" incubation in suitable cell culture system (e.g. for medium-scale transient protein expression of up to approx. 2×10^8 cells): Collect the cell suspension from the microplate wells to a sterile cell culture tray using a multichannel pipette. Transfer cell suspension to an appropriate cell culture system and adjust seeding density to desired concentration.

Note

For incubations in 30 ml volume scale please refer to Amaxa's™ Reference Guideline "Transient Protein Production using Nucleofector™ Technology". For incubations in higher batch volumes than 100 ml, incubation in a stirrer flask system or bioreactor typically is required. Please refer to culture conditions provided by supplier of cells.

3.2 Incubate the cells in humidified 37°C/5 % CO_2 incubator according to informations from cell supplier

3.3 Protein expression should be analyzed at different times. Depending on the protein, expression is often detectable after 4–8 hours. Maximal protein yield is usually between 1 and 7 days post Nucleofection™

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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