BioResearch

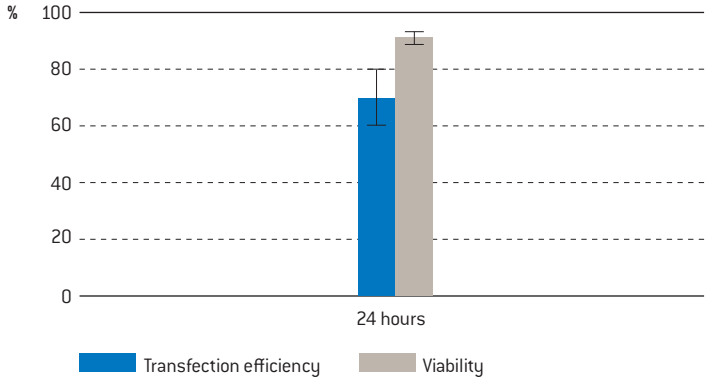
Lonza

Amaxa™ 96-well Shuttle™ Protocol for HeLa Cells (ATCC®)

Cell Description

Human cervix adenocarcinoma, adherent epithelial cell line ; (ATCC® CCL-2™).

Example for Nucleofection of HeLa Cells



Transfection efficiency and viability of HeLa cells 24 hours post Nucleofection™. HeLa cells were transfected with program 96-CN-114 and 0.4 µg of pmaxGFP™. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option. Cell viability was determined as % PI negative cells.

Product Description

Recommended Kits

SE Cell Line 96-well Nucleofector™ Kit

Cat. No.	V4SC-1096
Size (reactions)	1×96
SE Cell Line 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SC-1960
Size (reactions)	10×96
SE Cell Line 96-well Nucleofector™ Solution	22.5 ml
Supplement	5.0 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	10

Storage and Stability

Store Nucleofector™ Solution, Supplement and pmaxGFP™ at 4°C. For long term storage pmaxGFP™ is ideally stored at -20°C. The expiry 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 Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ plates
- 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) or Matrix TallTips™ (Matrix Technologies Corp., Cat. No. 7281). Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ wells without getting stuck
- For trypsinization: 0.5 mg/ml Trypsin and 0.2mg/ml EDTA in PBS and supplemented culture media or PBS/0.5 % BSA
- Appropriate volume of culture media at 37°C (255 µl per sample*); Minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 90 % (ATCC, Cat. No. 30-2003); fetal bovine serum, 10 % (Sigma, Cat. No. F-0643)
- 96-well culture plates or culture plates of your choice
- 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
- Appropriate number of cells (2×10^5 cells per sample). Minimal cell number: 2×10^4 (may result in slightly reduced viability; when using lower cell numbers viability is strongly decreased)

1. Pre Nucleofection™

Cell culture recommendations

1.1 Seeding conditions: $2–3\times10^3$ cells/cm^2
1.2 Replace media 2–3 per week (20–30 ml medium per 162 cm^2 flask)
1.3 Passage cells after reaching 90 % confluency
1.4 Cells should be passaged 3 days before Nucleofection™ depending on growth rate
1.5 Optimal confluency before Nucleofection™: 70–80 %

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.7 For harvesting the cells incubate the cells at 37°C with 0.5 mg/ml trypsin/0.2 mg/ml EDTA
1.8 Inactivate trypsinization reaction with supplemented culture media or PBS/0.5 % BSA

2. Nucleofection™

One Nucleofection™ Sample Contains

- 2×10^5 cells
- 0.2–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ or 30–300 nM siRNA (0.6–6 pmol/sample)
- SE Cell Line 96-well Nucleofector™ Solution

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 Manual "Nucleofector™ 96-well Shuttle™ System")
2.3 Select the appropriate Nucleofector™ program **96-CN-114**
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 175 µl* (see comments 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 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ DNA. For siRNA experiments we recommend to start using 30 nM–300 nM siRNA (0.6–6 pmol/sample). Depending on cell type, the minimum effective siRNA concentration can range between 1 nM and 1 µM. To validate optimal conditions for down regulation we recommend performing a time course (mRNA: 12–72 hours, protein/phenotype: 24–96 hours) in addition
2.7 Harvest the cells by trypsinization (please see 1.6–1.8)
2.8 Count an aliquot of the trypsinized cells and determine cell density
2.9 Centrifuge the required number of cells (2×10^5 cells per sample) at 90×g for 10 minutes at room temperature

2.10 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. Make sure the sample covers the bottom of the well, if necessary gently tap the Nucleocuvette™ plate. Avoid air bubbles while pipetting.

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 (please refer to Manual) or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (please refer to Manual)

2.13 After retainer opening, carefully remove the 96-well Nucleocuvette™ plate from the retainer.

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

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 Incubate the cells in a humidified 37°C/5 % CO_2 incubator until analysis. Gene expression is often detectable after only 4–8 hours

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

Technical Assistance and Scientific Support

USA/Canada
Tel 800 521 0390 (toll-free)
Fax 301 845 8338
scientific.support@lonza.com

Europe and Rest of World
Tel +49 221 99199 400
Fax +49 221 99199 499
scientific.support.eu@lonza.com

www.lonza.com

Lonza Cologne GmbH – 50829 Cologne, Germany