BioResearch

Lonza

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

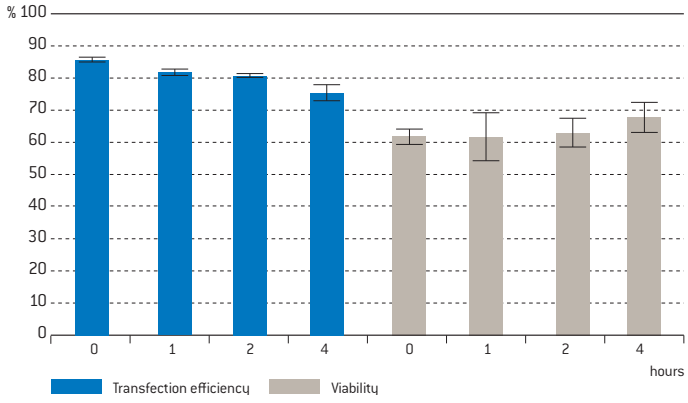
Cell Description

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

Note

This optimized protocol is designed for the use with automated systems where a prolonged storage of cells (up to 6 hours) in Nucleofector™ Solution is required prior to the Nucleofection™ Process.

Example for Nucleofection™ of HeLa



Transfection efficiency and viability depending on the incubation time pre Nucleofection™. HeLa cells (ATCC® CCL-2™) were transfected with program 96-CN-155 and 0.4 µg pmaxGFP™ Vector using the 96-well Shuttle™. At 24 hours post Nucleofection™, cells were analyzed on a FACSCalibur™ with HTS option. Cell viability was determined as number of % PI negative cells relative to the non-transfected control.

Product Description

Cat. No.	VHCA-4004
Size (reactions)	customized
Kit components per 1 x 96 unit:	
*Volumes will be customized depending on the required overfill to cover void volumes etc.	
Cell Line 96-well Nucleofector™ Solution AD1	1.035 ml*
Cell Line 96-well Nucleofector™ Solution AD2	1.265 ml*
Supplement	0.23 ml*
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate	1

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 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 mix the entire Nucleofector™ Solutions AD1 and AD2 with supplement directly before the experiment. If the solutions are supposed to be used partially i.e. to split up the experiment, mix Nucleofector™ Solution AD1, AD2 and supplement in a ratio of 1.035 / 1.265 / 0.23 ml.

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented and combined Nucleofector™ Solution AD1 and AD2 equilibrated to room temperature
- Supplied Nucleocuvette™ Plates
- 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 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® we always use here (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
- For trypsinization 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS
- Culture medium: 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%
- Prewarm appropriate volume of culture medium to 37°C (255 µl per sample; *see note at end of chapter 2)
- Appropriate number of cells (5 x 10^4 cells per sample; lower cell numbers may influence transfection results)

1. Pre Nucleofection™

Culturing of the cells

1.1 Do not use for Nucleofection™ cells after passage 10
1.2 Replace medium 3 times a week
1.3 Passage cells 2–3 times a week/passage cells every second to third day
1.4 Seed out 2 x 10^4 cells/cm^2
1.5 Subculture 2–3 days before Nucleofection™ with a ratio of 1 : 5–1 : 6

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 e.g. 0.5 mg trypsin/0.2 mg EDTA in PBS
1.8 Inactivate trypsinization reaction with supplemented culture media or PBS/0.5% BSA

2. Nucleofection™

One Nucleofection™ Sample Contains

- 5 x 10^4 cells
- 0.4–1 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 96-well Nucleofector™ Solution AD1+2

2.1 Please make sure that the Nucleofector™ Solutions and supplement are mixed in the appropriate ratio
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 Nucleofector™ Program **96-CN-155**
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) per 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
2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30 to 300 nM siRNA (0.6–6 pmol/sample)
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 (5 x 10^4 cells per sample) at 90xg for 10 minutes at room temperature
2.10 Resuspend the cell pellet carefully in 20 µl room temperature 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. Make sure the sample covers the bottom of the well, if necessary gently tap the Nucleocuvette™ Plate. Avoid air bubbles while pipetting.

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

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

2.13 Start 96-well Nucleofection™ Process by either:
- **A** pressing "Upload and start" in the 96-well Shuttle™ Software (please refer to Manual)
- **B** or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (please refer to Manual)

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

2.15 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

2.16 Plate desired amount of cells in culture system of your choice

*** 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 humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable after only 4–8 hours

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Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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