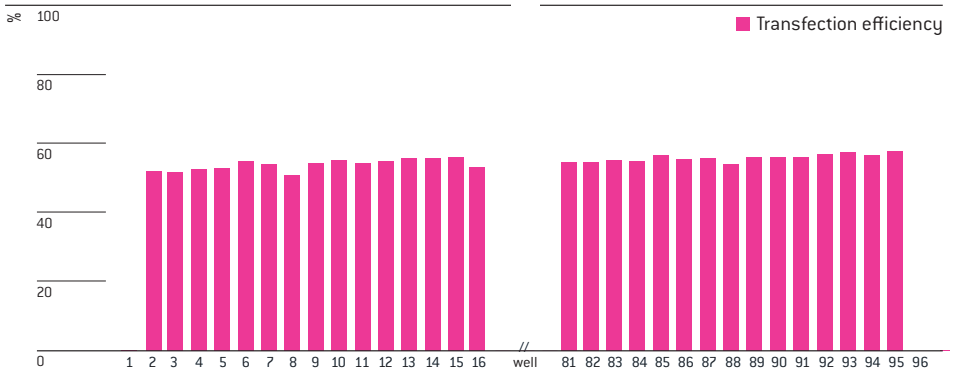
Amaxa® Cell Line 96-well Nucleofector® Kit SF

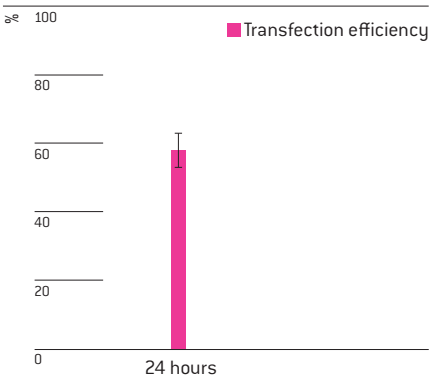
For HL-60

Human acute promyelocytic leukemia; myeloblastic cells

Example for Nucleofection® of HL-60 cells



Well-to-well uniformity of reporter gene expression after 96-well Nucleofection® of HL-60 cells. HL-60 cells were transfected with 0.4 pmaxGFP® Vector using the Cell Line 96-well Nucleofector® Kit SF. 24 hours post Nucleofection®, cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Wells without GFP expression are negative controls of cells in 96-well Nucleofector® Solution and plasmid DNA, but without Nucleofection®.



Transfection efficiency of HL-60 cells 24 hours post Nucleofection®. HL-60 cells were transfected with program 96-EN-138 and 0.4 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability (% PI negative cells) is usually around 60% after 24 hours.

Product Description

Cat. No.	VHCA-1002	VHCA-2002
Size (reactions)	1 x 96	10 x 96
Cell Line 96-well Nucleofector® Solution SF	2.025 ml	20.25 ml
Supplement	0.45 ml	4.5 ml
pmaxGFP® Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	45 µg	45 µg
Nucleocuvette® plate(s)	1	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 and standard Nucleofector® Solutions are not compatible.**

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 pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- Supplied Nucleocuvette® Plate(s)
- 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**: Iscove's Modified Dulbecco's Medium wih 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 80% ; fetal calf serum, 20%
- Prewarm appropriate volume of culture medium to 37°C (230 µl per sample)
- Appropriate number of cells (4 x 10^5 cells per sample; 1 x 10^5 cells can be used with slightly reduced viability. At even lower cell numbers viability is strongly decreased

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media 2 – 3 times a week
1.2 Passage cells at a density of 7 – 8 x 10^5 cells/ml. Do not use cells after passage 25 for Nucleofection®
1.3 Seed out 1 x 10^5 cells/ml
1.4 Subculture 3 days before Nucleofection®
1.5 Cells should be grown to a density of 5 – 7 x 10^5 cells/ml before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

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

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-EN-138**
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 150 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 **0.2 – 1 µg** plasmid DNA or 0.4 µg pmaxGFP® Vector or **30 nM – 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 (4 x 10^5 cells per sample) at 90xg 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 Incubate Nucleocuvette plate 10 min at room temperature

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. Recommendation for 96-well plates: Transfer 50 µl of resuspended cells to 150 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 humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours

Additional Information

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

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