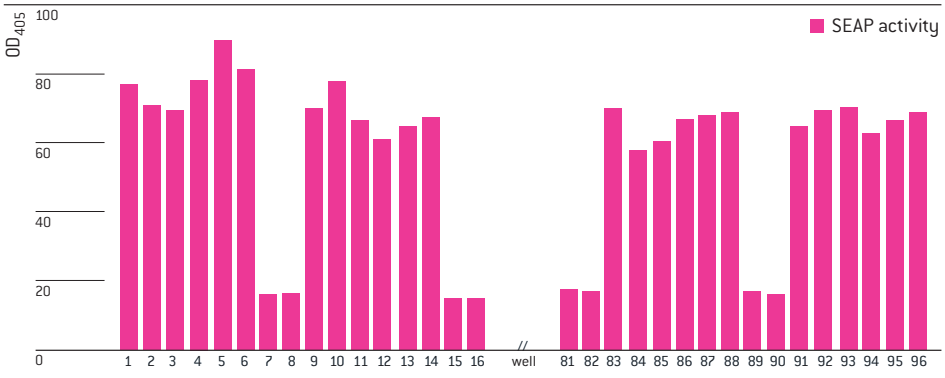
# Amaxa® Cell Line 96-well Nucleofector® Kit SF

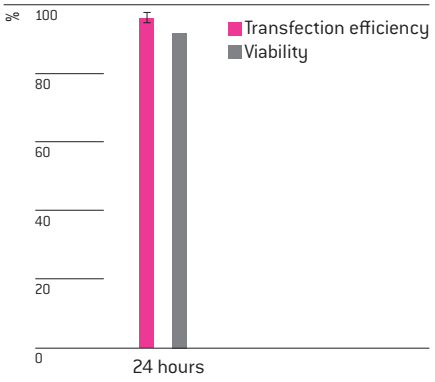
## For HepG2

Human hepatocellular carcinoma; adherent epithelial cells

### Example for Nucleofection® of HepG2 cells




**Well-to-well uniformity of reporter gene expression after 96-well Nucleofection® of HepG2 cells.** 2 x $10^5$ HepG2 cells were transfected with 0.4 µg of a plasmid encoding a secreted version of human placental alkaline phosphatase (SEAP) using the Cell Line 96-well Nucleofector® Kit SF. 24 hours post Nucleofection®, alkaline phosphatase activity of cell culture supernatants was measured (n=72, SD = ± 10% from mean). Wells without SEAP enzyme activity are negative controls of cells in 96-well Nucleofector® Solution and plasmid DNA, but without Nucleofection®.




**Transfection efficiency of HepG cells 24 hours post Nucleofection®.** 2 x $10^5$ HepG2 cells were transfected with program 96-EH-100 and 0.4 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability (CellTiter-Blue® cell viability assay) is approximately 93% 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 Nucleocuvette® Plate(s)
- Supplied pmaxGFP® Vector
- 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
- **For detaching cells**: We recommend using Accutase [PAA, Cat. No.: L11-007]
- **Culture medium**: complete growth medium Eagle's Minimal Essential medium adjusted to contain 1.5g/l sodium bicarbonate and supplemented with Earle's BSS, 2 mM L-glutamine(EMEM), 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids and 10% fetal bovine serum
- Prewarm appropriate volume of culture medium to 37°C (330 µl per sample)
- Appropriate number of cells (2 x $10^5$ cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection®

### Cell culture recommendations

1.1 Replace media every 3 – 4 days
1.2 Passage cells 2 times a week. We recommend using cells maximally to P19
1.3 Maintain cultures between 2 to 2.7 x $10^7$ cells /T162 flask, split ratio 1 : 3 – 1 : 4
1.4 Seed out 6.5 x $10^6$ cells/T162 flask
1.5 Subculture 3 days before Nucleofection®

### Accutase treatment

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, incubate the cells ~10 – 15 minutes at 37°C with Accutase solution (please see required material)
1.8 Allow cells to detach at 37°C/5% $CO_2$ for 10 – 15 minutes (do not exceed incubation time). Add fresh medium to detached cells and resuspend them

## 2. Nucleofection®

**One Nucleofection® Sample contains**

| |
|---|
| 2 x $10^5$ cells |
| 0.4 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-EH-100**
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 Harvest the cells by Accutase treatment (please see 1.6 – 1.8)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (**2 x $10^5$ cells per sample**) at **200xg for 10 minutes** at room temperature. Remove supernatant completely
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. 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 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)

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

2.15 Incubate Nucleocuvette® Plate for additional 10 minutes at room temperature

2.16 Resuspend cells with 180 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

2.17 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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