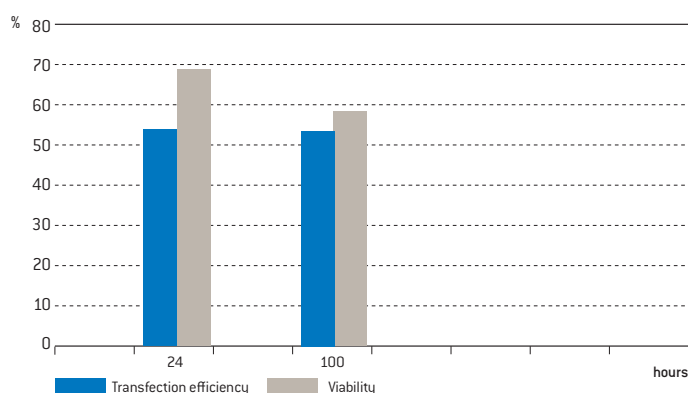


Amaxa™ 96-well Shuttle™ Protocol for Human Hepatocytes

Cell Description

Freshly isolated or cryopreserved human hepatocytes; polygonal, adherent cells.

Example for 96-well Nucleofection™ of human Hepatocytes



Transfection performance of human hepatocytes cells 24 hours and 120 hours post Nucleofection™. 1×10^5 cells were transfected with program 96-EX-147 using $1 \mu\text{g}$ pmaxGFP™ Vector, cells were seeded in one well of a 96-well plate. Cells were analyzed 24 and 120 hours post Nucleofection™ by counting. Cell viability was analyzed by using the CellTiter-Blue® cell viability assay (Promega Cat. No.: 68080).

Product Description

Recommended Kit(s) – P3 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-3096
Size (reactions)	1×96
P3 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0)	50 μg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SP-3960
Size (reactions)	10×96
P3 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1.0 $\mu\text{g}/\mu\text{l}$ 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 Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate
- 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
- Thawing medium: InVitroGRO CP Medium (InVitro Technologies; Cat. No. Z99029) and 2,22% Torpedo Antibiotic Mix (InVitro Technologies; Cat. No. Z99000). For correct volume addition of antibiotic mix refer to manufacturer's details
- Culture medium: HCM™ BulletKit® [Lonza; Cat. No.: CC-3198]
- Maintenance medium (for induction studies): William's E Medium [Lonza; Cat. No. 12-761F] without fetal bovine serum, supplemented with 1% Pen/Strep, 2 mM L-glutamine, 100 nM human recombinant insulin (Sigma; Cat. No. I-9278) and 100 nM dexamethasone (Sigma; Cat. No. D-1756)
- Ficoll solution: Culture medium containing 25% Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]
- For post Nucleofection™ culture: Collagen-coated 96-well culture plates (e.g. Collagen Type I CELLCOAT® 96-well plates, Greiner-bio-one; Cat. No.: 655950)
- Prewarm appropriate volume of medium to 37°C (150 µl per sample)
- Appropriate number of cells (1 x 10⁵ cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note

Transfection results for primary hepatocytes may be donor-dependent.

Thawing of cryopreserved human hepatocytes

Note

For fresh hepatocytes continue with step 1.5 of the thawing procedure.

- 1.1 Check your water bath with a mercury thermometer for the right temperature (temperature: 37°C ± 0.5°C)
- 1.2 Transfer your cells from the nitrogen vapour phase directly into the water bath. Stir the vial gently until the cells are completely thawed
- 1.3 Transfuse the cells directly into 43 ml of prewarmed thawing medium. To transfer cells completely, wash the vial with 5 ml of thawing medium. Combine both portions of thawing medium to reach a final volume of 48 ml
- 1.4 Resuspend the cells in the thawing medium by gently inverting the tube
- 1.5 Centrifuge the cells 5–7 minutes at 500–600 rpm (50–75xg) at room temperature. We recommend performing all centrifugation steps prior to Nucleofection™ in round bottom 2 ml reaction vials or 50 ml Flacon tubes. Centrifugation in conical 1.5 ml vials may lead to compact cell pellets which are difficult to resuspend.
- 1.6 Resuspend cells with great care by gently inverting the tube
- 1.7 Decant the supernatant into a waste tank except approx. 2 ml Resuspend the pellet in the residual supernatant by swivelling the tube
- 1.8 Top up with thawing medium to a final volume of 5 ml

2. Nucleofection™

One Nucleofection™ Sample Contains

- 1 x 10⁵ cells
 - 0.8–1 µg plasmid DNA (in 1–2 µl H₂O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300nM siRNA (0.6–6 pmol/sample)
 - 20 µl P3 Primary Cell 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 96-well Nucleofector™ Program **96-EX-147**
 - 2.4 Prepare 96-well cell collagen coated culture plates by filling appropriate number of wells with 100 µl of culture medium and equilibrate plates in a humidified 37°C/5% CO₂ incubator

Note

We recommend filling the outer wells with PBS instead of cells. In some cases evaporation of medium in these wells lead to edge effects during post nucleofection analysis.

- 2.5 Pre-warm an aliquot of culture medium to 37°C (50 µl per sample* see note at the end of this chapter)
- 2.6 Prepare 0.8–1.0 µg plasmid DNA or 1.0 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30 nM–300 nM (0.6–6 pmol/sample)
- 2.7 Pre-warm the supplemented Nucleofector™ Solution to room temperature
- 2.8 Take an aliquot of the thawed cell suspension and count the cells with trypan blue (1:2) to determine the cell number as well as cell viability
- 2.9 Centrifuge the required number of cells (1 x 10⁵ cells per well) at 500–600 rpm (50–75xg) for 5–7 minutes
- 2.10 Decant the supernatant completely so that no residual medium covers the cell pellet
- 2.11 Every resuspension step should be performed by gently swirling the tube not by pipetting as the cells are quite fragile
- 2.12 Resuspend the cell pellet 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. 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.

- 2.13 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
- 2.14 Place the lid on the 96-well Nucleocuvette™ Plate
- 2.15 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well “A1” must be in upper left position
- 2.16 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)
- 2.17 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer.
- 2.18 Incubate Nucleocuvette™ Plate for 10 min at room temperature
- 2.19 Fill up each well of the Nucleocuvette™ Plate with 180 µl Ficoll solution
- 2.20 Centrifuge cells in the Nucleocuvette™ Plate for 4 minutes at 75xg (deactivate the brake)
- 2.21 Remove 150 µl of the supernatant with great care (do not resuspend the hepatocytes in the pellet) and discard it

Note

This step separates the healthy hepatocytes from the dead ones and from cell debris. This step improves long term viability of the cells.

- 2.22 Add 50 µl prewarmed culture medium and resuspend the cells carefully
- 2.23 Plate desired amount of cells in culture system of your choice (96-well to 12-well plate). Recommendation: Transfer all of resuspended cells to 100 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 cells in a humidified 37°C/5% CO₂ incubator in culture medium for approx. 4 hours
- 3.2 Carefully remove the culture medium and replace it with 100 µl fresh culture medium (for 96-well culture plates)
- 3.3 Allow the transfected hepatocytes to recover in culture medium for at least three days before starting induction studies

Note

For induction studies (e.g. CYP3A4) replace the culture medium by maintenance medium after three days (72 hours) and induce Cyp expression by e.g. Rifampicin for additional 2 days.

- 3.4 Following Nucleofection™, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 8–16 hours. If this is not the case, the incubation period may be prolonged

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

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