BioResearch

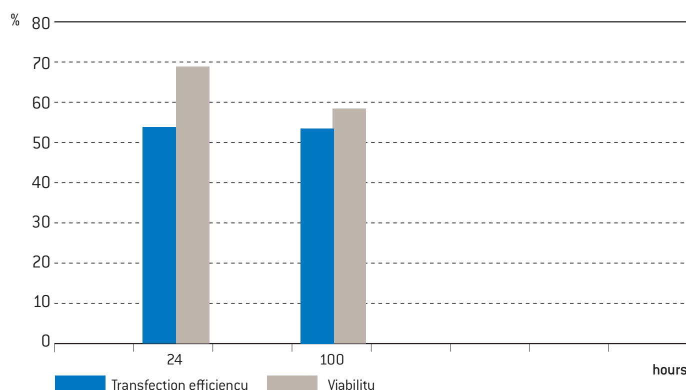
Lonza

Amaxa™ HT Nucleofector™ protocol for human hepatocytes

Cell description

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

Example for Nucleofection™ of human hepatocytes



Transfection performance of human hepatocytes cells 24 hours and 120 hours post Nucleofection™. 1 x 10^5 cells were transfected with program EX-147-AA using 1 µg 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.: G8080).

Product description

Recommended Kit(s)—P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2×384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-30102
Size (reactions)	10×384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well 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

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, the 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.

- HT Nucleofector™ system
- Supplemented Nucleofector™ solution at room temperature
- Supplied 384-well 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 384-well culture plates (e.g. Collagen Type I CELLCOAT® 96-well plates, Greiner-bio-one; Cat. No.: 655950)
- Pre-warm appropriate volume of medium to 37°C (186 µl per sample)
- Appropriate number of cells (1×10^5 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 Transfer 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

- 1×10^5 cells
- 0.8–1 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 P3 primary cell HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution
2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to the HT Nucleofector™ manuals)
2.3 Select the appropriate HT Nucleofector™ program **EX-147-AA**
2.4 Prepare 96-well cell collagen coated culture plates by filling appropriate number of wells with 146 µl of culture medium and equilibrate plates in a humidified 37°C/5% CO_2 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 (40 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 10^5 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 384-well Nucleocuvette™ plates

B. Multiple substrates (e.g. library transfection).

– Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
– Add 2 µl substrates (maximum) to each well
– Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
2.14 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1"must be in upper left position
2.15 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)
2.16 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel
2.17 Incubate Nucleocuvette™ plate for 10 minutes at room temperature
2.18 Fill up each well of the Nucleocuvette™ plate with 180 µl Ficoll solution
2.19 Centrifuge cells in the Nucleocuvette™ plate for 4 minutes at 75xg (deactivate the brake)
2.20 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.

2.21 Add 40 µl prewarmed culture medium and resuspend the cells carefully
2.22 Plate desired amount of cells in culture system of your choice (96-well to 12-well plate). Recommendation: Transfer all of resuspended cells to 140 µl pre-warmed medium prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 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.

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3. Post Nucleofection™

3.1 Incubate cells in a humidified 37°C/5% CO_2 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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