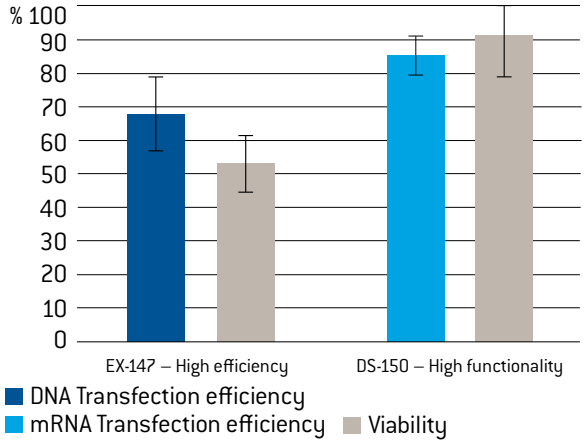
Bioscience Solutions

4D-Nucleofector™ Protocol for Human Hepatocytes

For 4D-Nucleofector™ X Unit–Transfection in suspension

For Cryopreserved Human Hepatocytes, Plateable, Induction Qualified [Lonza; Cat. No. HUCPI]; might be also suited for other freshly isolated or cryopreseved human hepatocytes; polygonal, adherent cells



Example for Nucleofection of cryopreserved plateable human hepatocytes

Transfection performance of Human Hepatocytes, Cryopreserved, Plateable and Induction Qualified (Lonza; cat. No.: HUCPI). **Left:** 5x 10^5 cells were transfected with program EX-147 (high efficiency; for short-term experiments) using 5 µg pmaxGFP™ Vector in 100 µL Nucleocuvette™ Vessel. Transfection efficiency and viability was analysed on day 1 post Nucleofection by manual counting of fluorescent cells and CellTiter-Blue® Cell viability assay (Promega Cat. No.: G8080).
Right: 5x 10^5 cells were transfected with program DS-150 (high functionality; for long-term experiments) using 5 µg CleanCap™ mCherry mRNA (TriLink; Cat. No.: L-7203) in 100 µL Nucleocuvette™ Vessel. Cells were analyzed for transfection efficiency on day 7 post Nucleofection by manual counting of fluoreoscent cells. Cell viability was analyzed by using the CellTiter-Blue® cell viability assay (Promega Cat. No.: G8080). Expression of the mRNA as well as cell viability were sustained between day 1 and 7.

Product Description

Recommended Kit(s) – P3 Primary Cell 4D-Nucleofector™ X Kit

Cat No.	V4XP-3012	V4XP-3024	V4XP-3032
Transfection volume	100 µL	100 µL	20 µL
Size [reaction]	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 mL (0.492 mL + 27% overfill)	2.25 mL (1.968 mL + 13% overfill)	0.675 mL (0.525 mL + 22% overfill)
Supplement	2 x 0.15 mL (0.108 mL + 27% overfill)	0.5 mL (0.432 mL + 13% overfill)	0.15 mL (0.115 mL + 22% overfill)
pmaxGFP™ Vector (1 µg/µL in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µL)	12	24	-
16-well Nucleocuvette™ Strips (20 µL)	-	-	2

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

4D-Nucleofector™ Solutions can only be used with conductive polymer Nucleocuvette™ Vessels, i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ 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 prior to use. For preparing aliquots, mix Nucleofector™ Solution and Supplement in a ratio of 4.5 : 1 (see Table 1).

- 4D-Nucleofector™ System (4D-Nucleofector™ Core and X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µL single Nucleocuvette™ or 20 µL 16-well Nucleocuvette™ Strips
- Compatible tips for 20 µL Nucleocuvette™ Strips: 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
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µL

Note
When using pmaxGFP™ Vector as positive control, dilute the stock solution to an appropriate working concentration that allows pipetting of the recommended amounts per sample (see Table 3). Make sure that the volume of substrate solution added to each sample does not exceed 10% of the total reaction volume (2 µL for 20 µL reactions; 10 µL for 100 µL reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260:A280 ratio should be at least 1.8
- Cell culture plates of your choice
- **Thawing medium:** Human Cryopreserved Hepatocytes Thawing Medium (Lonza; Cat. No.: MCHT50)
- **Plating medium:** Hepatocyte Plating Media w/ Supplement (Cat. No.: Lonza; MP100 or MP250)
- **Culture medium:** HCM™ BulletKit™ (Lonza; Cat. No.: CC-3198)
- For sandwich culture: Corning® Matrigel® Matrix Phenol Red-Free, LDEV-Free, 10 mL, (Corning; Cat. No. 356237)
- For post Nucleofection culture: Collagen-coated culture plates (e.g. Collagen Type I coated plates of your choice)
- Prewarm appropriate volume of culture medium to 37°C (see Table 2) Appropriate number of cells/sample (see Table 3)

1. Pre Nucleofection

Note
Transfection results on primary human hepatocytes may be donor-dependent.

Thawing of cryopreserved human hepatocytes

Note
- For fresh hepatocytes continue with step 1.6 of the thawing procedure
- Thawing procedure according to Lonza recommendation for Human Hepatocytes, Cryopreserved, Plateable and Induction Qualified with small adaptations

1.1 Check your water bath with a thermometer for the right temperature (temperature: 37 °C ± 0.5 °C). Pre-warm the thawing medium and plating medium

Note
For the next steps, work as quickly and as efficiently as possible to maintain the viability of cells.

1.2 Transfer your cells from the nitrogen vapour phase directly into the water bath

1.3 Thaw the vial for approximately 90–120 seconds. The vial will thaw from the outside to the inside. You can see a spindle form and shrink as the vial thaws. When almost completely thawed and only a small spindle of frozen cells remains, remove vial from water bath

1.4 Quickly remove vial cap and carefully pour or pipette (with a wide-bore tip) hepatocytes into the 50 mL conical tube of appropriate warmed thawing medium

1.5 Pipette approximately 1 mL thawing medium back into the original vial and pour or pipette the remaining cells back into the 50 mL tube of thawing medium to ensure that all hepatocytes are transferred. Suspend the cells by carefully rocking the 50 mL tube by hand, for a few seconds
Note: DO NOT VORTEX

1.6 Centrifuge the cells 8 minutes at 100 x g at room temperature.

1.7 Pour supernatant into a waste bottle, inverting completely, without shaking (or aspirate off supernatant carefully with a vacuum aspirator)

1.8 For each vial, gently resuspend cells in 3 mL plating medium

2. Nucleofection

For Nucleofection sample contents and recommended Nucleofector™ Program, please refer to Table 3.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
2.3 Select/Check for the appropriate Nucleofector™ Program (see Table 3)
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended palting media (see Table 2) 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 (see Table 2)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see Table 3)
2.7 Determine the viability and yield of your hepatocytes using the Trypan Blue exclusion method (according to "Technical Information & Instructions – Suspension and Plateable Cryopreserved Hepatocytes" available on Lonza website)
2.8 Centrifuge the required number of cells (see Table 3) at 80xg for 8 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 3)
2.10 Prepare mastermixes by dividing cell suspension according to number of substrates
2.11 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.12 Transfer mastermixes into the Nucleocuvette™ Vessels

Note
As leaving cells in 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™ Vessels to make sure the sample covers the bottom of the cuvette
2.14 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.15 Start Nucleofection process by pressing "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.16 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.17 Incubate Nucleocuvette™ Vessel 10 min at room temperature
2.18 Resuspend cells with pre-warmed plating medium (for recommended volumes see Table 2). Mix cells by gently pipetting up and down oncs. When working with the 100 µL Nucleocuvette™ Vessel use the supplied pipettes and avoid repeated aspiration of the sample
2.19 Plate desired amount of cells in culture system of your choice (for recommended volumes see Table 2)

3. Post Nucleofection

Place plate in a 37°C/5% CO_2 incubator. It is very important that cells are evenly dispersed in the wells. This can be accomplished by moving plate in a north-south-east-west motion, while maintaining contact with the incubator shelf. Repeat at 15, 30, 45, 60, 120, 180, 240 minutes post-seeding. Failure to evenly disperse cells after placing in the incubator can result in accumulation of cells in the center of each well.

Note
This should not be attempted with 96-well plates. To avoid a vortex effect, with cells accumulating in the center of wells, leave 96-well plate undisturbed in Biosafety cabinet for 2-3 minutes before carefully placing in cell culture incubator. This will allow time for the cells to settle on the bottom of the wells and they will be less likely to congregate in the center.

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator in plating medium for approx. 4 – 6 hours. Carefully remove the plating medium and replace it with fresh culture medium
3.2 An overlay with Matrigel® is recommended after 4 – 6 hours or at 24 hours after seeding. Please refer to "Technical Information & Instructions – Suspension and Plateable Cryopreserved Hepatocytes" available on Lonza website

Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to: **https://knowledge.lonza.com/** under the section citations

For more technical assistance, contact our Scientific Support Team:

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Table 1: Volumes required for a single reaction

	100 µL Single Nucleocuvette™	20 µL Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µL	16.4 µL
Volume of Supplement	18 µL	3.6 µL

Table 2: Required amounts of cells and media for Nucleofection

		100 µL Single Nucleocuvette™	20 µL Nucleocuvette™ Strip
Culture plate format		24-well plate	96-well plate
Plating medium	Added to sample post Nucleofection	400 µL plating medium	80 µL plating medium
Volume of sample transferred to culture plate		complete sample (use supplied pipettes)	complete sample
Final culture volume		500 µL	100 µL

Table 3: Contents of one Nucleofection sample and recommended program

		100 µL Single Nucleocuvette™	20 µL Nucleocuvette™ Strip
Cells		5×10^5 (Lower or higher cell numbers may influence transfection results)	1×10^5 (Lower or higher cell numbers may influence transfection results)
Substrate*	pmaxGFP™ Vector	5 µg	1 µg
or	plasmid DNA (in H_2O or TE)	1–5 µg	0.2–1.5 µg
or	siRNA	30–300 nM siRNA (3–30 pmol/sample)	30–300 nM siRNA (0.6–6 pmol/sample)
P3 4D-Nucleofector™ X Solution		100 µL	20 µL
Program		EX-147 (high efficiency) DS-150 (high functionality)	EX-147 (high efficiency) DS-150 (high functionality)

* Volume of substrate should comprise maximum 10% of total reaction volume

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