BioResearch

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4D-Nucleofector™ Protocol for General Instructions

For 4D-Nucleofector™ LV Unit

This protocol provides general instructions on how to perform an experiment with the 4D-Nucleofector™ LV Unit using either 1 mL or LV Nucleocuvette™ Cartridges. For cell-type specific protocol, please refer to our protocol database (www.lonza.com/protocols) or contact our Scientific Support Team.

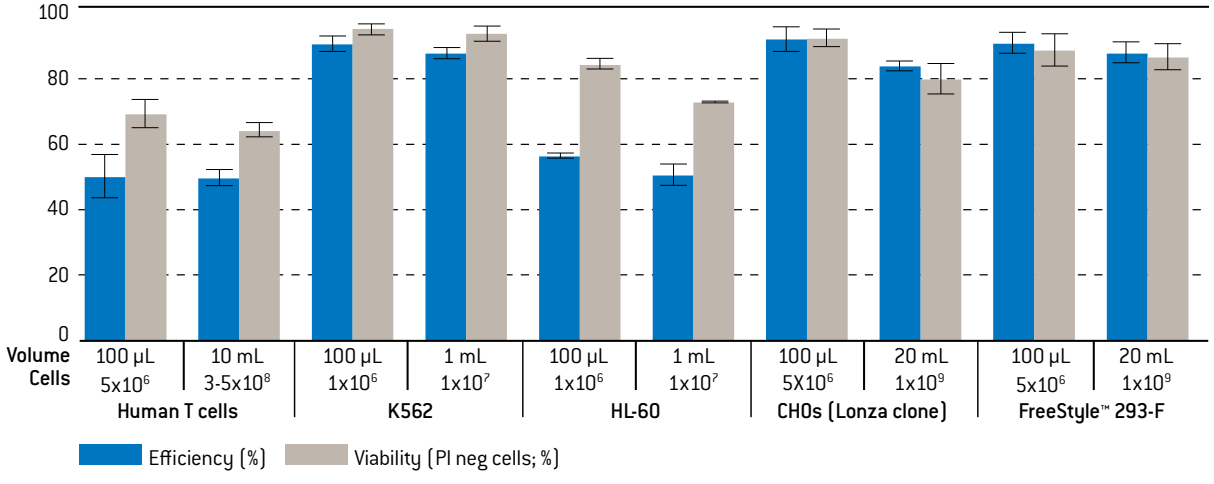
In general, before performing an experiment in the 4D-Nucleofector™ LV Unit it is recommended to establish your optimal setup in small scale using the 20 or 100 µL formats of the 4D-Nucleofector™ X Unit:

- Optimal Nucleofection conditions (Nucleofector™ Solution and Program according to Lonza optimized protocol or based on own optimizations)
- Optimal cell concentration
- Optimal substrate amount for the selected cell concentration. Conditions are transferable from the 4D-Nucleofector™ X Unit to the LV Unit by applying the same scaling factor for volume, cells and substrate.

Required Material

Here you will only find materials required in addition to those required for a 4D-Nucleofector™ X Unit experiment:

- 4D-Nucleofector™ LV Unit
- 4D-Nucleofector™ LV Kit with specific 4D-Nuclofector™ Solution and either 1 mL Nucleocuvette™ Cartridge or LV Nucleocuvette™ Cartridge
- For loading cell suspension into 1 mL Nucleocuvette™ Cartridges: Compatible tips, e.g. OneTouch pipet tips, 1250 µL (Sorenson™ BioScience Inc., Cat. No. 10370; via VWR, Cat. No. 89082-348)
- For working with LV Nucleocuvette™ Cartridges:
  - Supplied 4D-Nucleofector™ LV Reservoirs (alternatively you may use bags). Additional reservoirs can be ordered separately (Lonza, Cat. No. V4LR-1001)
  - Magnetic stirrer, e.g. MR Hei-Tec (Heidolph, Cat. No. 036110550)
  - BD Falcon™ cell strainer, 100 µm (BD, Cat. No. 352360)



Transferability from Small to Large Volume

Comparison of various exemplary cell types transfected with pmaxGFP™ Vector in small volume (100 µL Nucleocuvette™ Vessels) or larger volumes (1 mL Nucleocuvette™ Cartridge or LV Nucleocuvette™ Cartridge) using the same conditions. Transfection efficiency and cell viability were analyzed 24 hours post Nucleofection. Data represent the mean of various independent experiments.

– Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260:A280 ratio should be at least 1.8
– Cell culture vessels of your choice
– Culture medium: Use established culture media
– Prewarm appropriate volume of culture medium to 37°C
– Appropriate number of cells/sample (see Table 1)

1. Pre Nucleofection

Please follow your cell culture procedures established for transfecting these cells in smaller format (X Unit).

2. Nucleofection

For Nucleofection sample contents and recommended Nucleofector™ Program, please refer to Table 1.

Notes

– When working with large cell numbers consider the volume of the cell pellet before adding Nucleofector™ Solution. The total volume of cell pellet, Nucleofector™ Solution and substrate should match 1 mL (for 1 mL Nucleocuvette™ Cartridge) or up to 20 mL (for LV Nucleocuvette™ Cartridge).
– 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.

2.1 Working with 1 mL Nucleocuvette™ Cartridges

1. Please ensure that the entire supplement is added to the Nucleofector™ Solution
2. Start 4D-Nucleofector™ System and select respective vessel type (1 mL Nucleocuvette™ Cartridge)
3. Create or upload experimental parameter file (for details see device manual) and select the appropriate Nucleofector™ Solution and Program (see Table 1)
4. Prepare cell culture vessels with desired volume of recommended culture media and pre-incubate vessels in a cell culture incubator
5. Prepare required amount of substrate (see Table 1)
6. For adherent cells: Harvest the cells by trypsinization
7. Count an aliquot of the cells and determine cell density
8. Centrifuge the required number of cells (see Table 1) at 90xg for 10 minutes at room temperature. Remove supernatant completely
9. Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 1)
10. Add required amount of substrate (e.g. plasmid DNA, mRNA) to the sample. Ensure that the volume of substrate solution added to each sample does not exceed 10% of the total reaction volume (i.e. 100 µL for a 1 mL reaction)
11. Transfer cell suspension supplemented with substrate into 1 mL Nucleocuvette™ Cartridge using a 1 mL pipette
12. Insert Nucleocuvette™ Cartridge into the 4D-Nucleofector™ LV Unit. Ensure the Nucleocuvette™ Cartridge is correctly inserted
13. Start Nucleofection process by pressing “Start” on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
14. After run completion, carefully remove the Nucleocuvette™ Cartridge from the slot
15. Remove sample from Nucleocuvette™ Cartridge using a 1 mL pipette

Table 1: Contents of one Nucleofection Sample and Recommended Program

		Per 1 mL Reaction	Notes
Cells	Small cells (e.g. T cells)	$5 \times 10^7 - 2 \times 10^8$	Lower or higher cell numbers may influence transfection results
	Normal cells (e.g. CHO-S)	$1 \times 10^7 - 1 \times 10^8$	
	Large cells (e.g. HEK293)	$1 \times 10^7 - 5 \times 10^7$	
Substrate*	pmaxGFP™ Vector	40 µg	Volume of substrate added should not exceed 10% of total reaction volume to avoid significant dilution of the Nucleofector™ Solution by substrate buffer. Alternatively, the substrate might be diluted in Nucleofector™ Solution.
or	plasmid DNA	10 – 80 µg	
or	mRNA	Titrate optimal amount	
or	siRNA	30 – 300nM siRNA (30 – 300 pmol/mL	
Optimal 4D-Nucleofector™ LV Solution		1 mL	
Program		Use established one	

16. Optional: In case a post-incubation step is used for Nucleofection of the cells in small volume, transfer sample into an Eppendorf tube and incubate transfected sample for 10 min at room temperature
17. Pipet sample into culture vessel containing pre-warmed medium

2.2 Working with LV Nucleocuvette™ Cartridges

Before preparing the cells mount an LV Nucleocuvette™ Cartridge into the 4D-Nucleofector™ LV Unit (for details, please refer to the device manual).

1. Please ensure that the entire supplement is added to the Nucleofector™ Solution
2. Start 4D-Nucleofector™ System and select respective vessel type (LV Nucleocuvette™ Cartridge)
3. Create or upload experimental parameter file (for details see device manual) and select the appropriate Nucleofector™ Solution and Program (see Table 1)
4. Prepare cell culture vessels with desired volume of recommended culture media and pre-incubate vessels in a cell culture incubator
5. Prepare required amount of substrate (see Table 1)

Preparing the cells:

6. For adherent cells: Harvest the cells by trypsinization
7. Count an aliquot of the cells and determine cell density
8. Centrifuge the required number of cells (see Table 3) at 90xg for 10 minutes at room temperature. A higher centrifugation velocity may be required when using larger centrifugation vessels (e.g. 300xg for 500 ml). Ensure the cells are pelleted within 10 minutes.
9. Remove supernatant completely
10. Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 1)
11. Pipette cell suspension through a cell strainer into a Falcon tube
12. Loading the inlet reservoir(s):
    a. **When working with one inlet reservoir:** Add required amount of substrate (e.g. plasmid DNA, mRNA)to the cell suspension; ensure that the volume of substrate solution added to each sample does not exceed 10% of the total reaction volume (i.e. 1 ml for a 10 mL reaction) to avoid significant dilution of the Nucleofector™ Solution by substrate buffer
    b. **When working with two separate inlet reservoirs:** Keep substrate separately and optionally dilute it in Nucleofector™ Solution. The ratio of cell suspension to substrate should be kept between 1:1 to 10:1 to allow proper mixing. For ratios other than 1:10, please dilute substrate in Nucleofector™ Solution
13. Mount the inlet reservoir(s) or bag(s) into the reservoir holder(s)
14. Immediately start stirring the cell suspension at ~300 rpm to prevent cell sedimentation. Ensure that the magnet is truly stirring
15. Remove red caps from the Spiros connectors on the inlet tubes of the cartridge and the blue caps from the reservoir tubes and connect the tubes
16. Start Nucleofection process by pressing “Start” on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
17. After run completion, carefully disconnect the outlet reservoir and transfer the cells in the culture system of your choice

3. Post Nucleofection

Incubate the cells in cell culture incubator.

Additional Information

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

For more technical assistance, contact our Scientific Support Team:

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