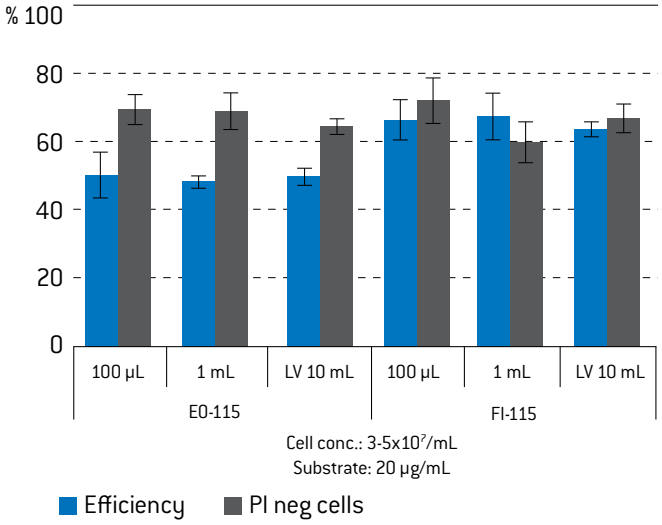
BioResearch

4D-Nucleofector™ Protocol for Unstimulated Human T Cells

For 4D-Nucleofector™ LV Unit

Unstimulated human T cells (small round lymphoblastoid cells) are a subpopulation of human peripheral blood mononuclear cells (PBMC).

Important: Before performing an experiment in the 4D-Nucleofector™ LV Unit it is recommended to establish optimal cell and substrate concentrations in small scale using the 4D-Nucleofector™ X Unit. Conditions are transferable from the 4D-Nucleofector™ X Unit to the LV Unit.



Example for Nucleofection of Human T Cells

Transfection efficiency and viability of human CD3⁺ T cells 24 hours post Nucleofection. Freshly isolated, unstimulated PBMCs (3-5 x 10^7 cells/mL) were transfected with pmaxGFP™ Vector (20 µg/mL) using programs EO-115 (for high viability/functionality) or FI-115 (for high efficiency) in 100 µL Nucleocuvette™ Vessels, in 1 mL Nucleocuvette™ Cartridges or with 10 mL in LV Nucleocuvette™ Cartridges. 24 hours post Nucleofection, transfection efficiency of CD3-positive T cells was analyzed on a FACSCalibur™ (Becton Dickinson). Cell viability was determined via propidium iodide (PI) staining. Data represent the mean of various independent experiments with different donors.

Product Description

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

Cat No.	V4LP-3002	V4LP-3020
Transfection volume	1 mL	1 – 20 mL
Size [reaction]	2	1
Nucleofector™ Solution	2.25 mL	22.5 mL
Supplement	0.5 mL	5 mL
1 mL Nucleocuvette™ Cartridge	2	-
LV Nucleocuvette™ Cartridge	-	1
4D-Nucleofector™ LV Reservoir	-	2

Storage and Stability

Store Nucleofector™ Solution and Supplement at 4°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, the 96-well Shuttle™ System or the HT 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 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 LV Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 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)
- 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 2)

1. Pre Nucleofection

Notes

- This protocol is designed for fresh unstimulated primary human T cells from whole PBMCs. Depending on application T cells can be further enriched
- Transfection results may be donor-dependent
- For preparation, do not perform protocols using hypo-osmolar buffers. This may lead to high cell mortality after Nucleofection
- For freshly isolated cells no cultivation is required prior to Nucleofection. For cryopreserved cells we recommend incubating the thawed cells for 1–2 hours at 37°C in culture medium before Nucleofection
- For Nucleofection of stimulated T cells, please refer to the Optimized Protocol for Stimulated Human T Cells

Preparation of cells

Isolate and optionally enrich cells according to your established protocols.

2. Nucleofection

For Nucleofection Sample contents and recommended Nucleofector™ Program, please refer to Table 2.

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)
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 2)
6. Select the appropriate Nucleofector™ Program (see Table 2)
7. Count an aliquot of the cells and determine cell density

8. Centrifuge the required number of cells (see Table 2) 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 2)
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 1mL 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
16. 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 2)
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 2)
6. Preparing the cells:
7. For adherent cells: Harvest the cells by trypsinization
8. Count an aliquot of the cells and determine cell density
9. Centrifuge the required number of cells (see Table 2) 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.
10. Remove supernatant completely
11. Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 2)
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

Table 1: Volumes required for a single reaction

	1 mL reaction
Volume of Nucleofector™ Solution	820 µL
Volume of Supplement	180 µL

Table 2: Contents of one Nucleofection Sample and Recommended Program

		Per 1 mL Reaction	Notes
Cells		1 x 10^7 - 5 x 10^7	Lower or higher cell numbers may influence transfection results
Substrate*	pmaxGFP™ Vector	20 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 (in H_2O or TE)	10 – 40 µg	
or	siRNA	Titrate optimal amount	
P3 4D-Nucleofector™ LV Solution		1 mL	
Program		FI-115 (for high efficiency) or E0-115 (for high unctionality)	

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

Until analysis, culture cells as usual in a cell culture incubator

4. 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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