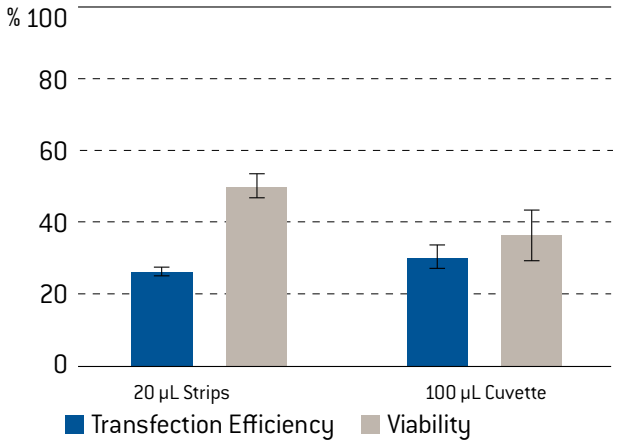
Bioscience Solutions

4D-Nucleofector™ Protocol for 3T3-L1 (adipocytes) Cells

For 4D-Nucleofector™ X Unit—Transfection in Suspension

Mouse embryonal fibroblast, differentiated into adipocytes; Fibroblast-like cells before differentiation; adipocyte-like cells after differentiation



Example for Nucleofection of 3T3-L1 (adipocytes)

Transfection efficiency and viability of 3T3-L1 (adipocytes) cells 24 hours post Nucleofection. 3T3-L1 (adipocytes) cells were transfected with program CA-133 in 20 µL Nucleocuvette™ Strips (0.8 µg pmaxGFP™ Vector) or 100 µL Nucleocuvette™ Vessels (4 µg pmaxGFP™ Vector). 24 hours post Nucleofection, transfection efficiency was analyzed on a FACSCalibur™ (Becton Dickinson). Cell viability was determined using ViaLight™ Plus Assay and normalized to untransfected control sample.

Product Description

Recommended Kit(s) – SE Cell Line 4D-Nucleofector™ X Kit

Cat No.	V4XC-1012	V4XC-1024	V4XC-1032
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™ and the 96-well Shuttle™ System. They are not compatible with the Nucleofector™ II/2b Device.

Required Material

Note
Please make sure that the 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.
- **For coating of plates**: Prepare a 2.5 mg/mL collagen stock solution by dissolving collagen (Type I; Sigma Cat. No. C-7661) in 0.2% sterile acetic acid. Stir at room temperature for 4 hours. Alternatively, prepare a 2% gelatin solution (20 mg/mL, gelatin solution type B from bovine skin [Sigma, Cat. No. G-1393])
- **For detaching cells:** 0.5 mg/mL Trypsin and 0.2 mg/mL EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- **Culture medium I**: DMEM [Lonza; BE 12-604F] supplemented with 10% calf bovine serum.
- **Culture medium II**: Preadipocyte basal medium-2 (500 mL) [Lonza; PT-8202] supplemented with FBS, L-glutamine and GA-1000 from the preadipocyte growth medium-2 SingleQuots™ Kit [Lonza; PT-8502]. The PGM™-2 BulletKit™ [Lonza; PT-8002] contains both, the basal medium and SingleQuots™ Kit
- Differentiation medium: 200 mL of culture medium with rhInsulin, dexamethasone, IBMX (3-isobuty-l-methyl-xanthine) and indomethacin from the preadipocyte growth medium-2 SingleQuots™ Kit [Lonza; PT-8502].
- Prewarm appropriate volume of culture medium to 37°C (see Table 2)
- Appropriate number of cells/sample (see Table 3)

1. Pre Nucleofection

Cell culture recommendations for undifferentiated 3T3-L1 cells

1.1 Subculture cells 3 times a week. Replace culture medium I every 2 – 3 days
1.2 Passage cells at 80% confluency. Avoid 100% confluency!
1.3 Seed out 6300 cells/cm^2

Differentiation

1.4 Cells should be seeded in culture medium II with a density of 6300 cells / cm^2
1.5 Let cells grow to 80 – 100% confluency within 2-3 days and start differentiation at this point.
1.6 To start differentiation change culture medium to differentiation medium (day 0 of differentiation)
1.7 On day 6 of differentiation renew differentiation medium
1.8 Cells should be differentiated for 10 days. For Nucleofection™ only use 10 day differentiated 3T3-L1.

Trypsinization

1.9 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.10 For harvesting, incubate the cells ~10 minutes at 37°C with indicated trypsinization reagent (please see required material)
1.11 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5% BSA once the majority of the cells (>90 %) have been detached

Preparation of Gelatin-coated plates

1.12 Prewarm a 2% gelatin solution at 37°C for 30 minutes
1.13 Then dissolve gelatin in PBS at 1 : 8 (final conc.: 2.5 mg/mL)
1.14 Add 0.2 mL per cm^2 of diluted gelatin to the culture vessel of your choice and incubate for 30 minutes at room temperature under laminar flow.
1.15 Exhaust non-coated gelatin and dry plates for about 15 minutes under a laminar flow.

Alternative: Preparation of collagen-coated 6-well plates for cultivation after Nucleofection™

1.16 Add 2 µL per cm^2 of collagen stock and 0.1 mL 30% ethanol per cm^2 to the culture vessel of your choice and incubate for 24 hours at room temperature under laminar flow.
1.17 Wash 2 x with PBS
1.18 Dry plates for about 15 minutes under a laminar flow

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 culture media (see Table 4) 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 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see Table 3)
2.7 Harvest the cells by trypsinization (please see 1.9–1.11)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see Table 3) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 3)
2.11 Prepare mastermixes by dividing cell suspension according to number of substrates
2.12 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.13 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

2.14 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.15 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.16 Start Nucleofection Process by pressing the “Start” on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.17 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.18 Resuspend cells with pre-warmed medium (for recommended volumes see Table 2). Mix cells by gently pipetting up and down two to three times. When working with the 100 µL Nucleocuvette™ 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

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours

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		6-well plate	96-well plate
Culture medium	Pre-filled in plate	1000 µL	175 / µL
	Added to sample post Nucleofection	400 µL	80 µL
Volume of sample transferred to culture plate		complete sample (use supplied pipettes)	25 µL
Final culture volume		1500 µL	200 µL

Table 3: Contents of one Nucleofection sample and recommended program

		100 µL Single Nucleocuvette™	20 µL Nucleocuvette™ Strip
Cells		4×10^6 (Lower or higher cell numbers may influence transfection results)	8×10^5 (Lower or higher cell numbers may influence transfection results)
Substrate*	pmaxGFP™ Vector	4 µg	0.8 µg
or	plasmid DNA (in H_2O or TE)	2–10 µg	0.4–2 µg
or	siRNA	30–300 nM siRNA (3–30 pmol/sample	30–300 nM siRNA (0.6–6 pmol/sample)
SE 4D-Nucleofector™ X Solution		100 µL	20 µL
Program		CA-133	CA-133

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

Additional Information

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

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