

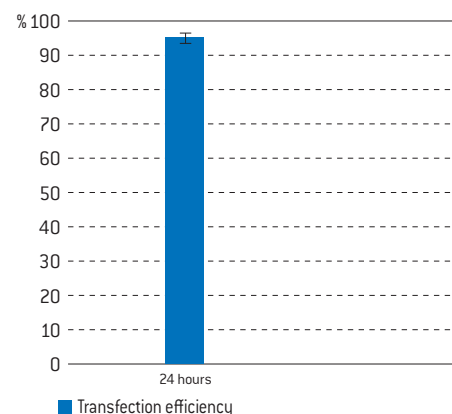
Amaxa™ 4D-Nucleofector™ Protocol for NIH/3T3 [ATCC®]

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

NIH Swiss mouse embryo; adherent fibroblastoid cells; [ATCC® CRL-1658™, cryopreserved]

Example for Nucleofection™ of NIH/3T3

Transfection efficiency of NIH/3T3 cells 24 hours post Nucleofection™. NIH/3T3 cells (ATCC® CRL-1658™) were transfected with program EN-158 and 0.4 µg of pmaxGFP™ Vector in 20 µl Nucleovette™ Strips. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ [Becton Dickinson]. Cell viability (% PI negative cells) is usually around 90 % after 24 hours.



Product Description

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

Cat. No.	V4XC-3012	V4XC-3024	V4XC-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 % overflow)	2.25 ml (1.968 ml + 13 % overflow)	0.675 ml (0.525 ml + 22 % overflow)
Supplement	2 x 0.15 ml (0.108 ml + 27 % overflow)	0.5 ml (0.432 ml + 13 % overflow)	0.15 ml (0.115 ml + 22 % overflow)
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 Nucleocuvettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. 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. The ratio of Nucleofector™ Solution to supplement is 4.5: 1 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ 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 1µg/µl**

Note

For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should 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 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:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/l sodium bicarbonate and 4.5 g/l glucose, 90 % [ATCC®, Cat. No. 30-2002]; bovine calf serum [ATCC®, Cat. No. 30-2030], 10 %. The medium and the bovine calf serum have a tremendous influence on both the transfection efficiency and cell survival. We strongly recommend to use medium and bovine calf serum from ATCC® for culture and Nucleofection™ Experiments
- Prewarm appropriate volume of culture medium to 37 °C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Cell culture recommendations

- 1.1 Replace media 2 times a week
- 1.2 Passage cells at 70–80 % confluency. The cell layer should not become completely confluent
- 1.3 Seed out 10^2 – 10^3 cells/cm²
- 1.4 Subculture 3 days before Nucleofection™
- 1.5 Optimal confluency for Nucleofection™: 70–80 %. Higher cell densities may cause lower Nucleofection™ Efficiencies

Trypsinization

- 1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
- 1.7 For harvesting, incubate the cells ~5 minutes at 37 °C with indicated trypsinization reagent (please see required material)
- 1.8 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5 % BSA once the majority of the cells (>90 %) have been detached

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 % CO₂ 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 1.6–1.8)
- 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 Incubate Nucleocuvette™ 10 minutes at room temperature
- 2.19 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). 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
- 2.20 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

3. Post Nucleofection™

- 3.1 Incubate the cells in humidified 37 °C/5 % CO₂ incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours

Additional Information

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

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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 medium per sample post Nucleofection™ (for transfer and culture)	1.5 ml	255 µl
Cell number per Nucleofection™ Sample	1 x 10 ⁶ [Lower or higher cell numbers may influence transfection results]	2 x 10 ⁵ [5 x 10 ⁴ cells can be used with slightly reduced viability. At even lower cell numbers viability is strongly decreased]

Table 3: Contents of one Nucleofection™ Sample and recommended program

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells	1 x 10 ⁶	2 x 10 ⁵
Substrate*	pmaxGFP™ Vector	2 µg
	or plasmid DNA (in H ₂ O or TE)	1–5 µg
	or siRNA	30–300nM siRNA (3–30 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ Solution	100 µl	20 µl
Program	EN-158	EN-158

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

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
6-well culture plate	1 ml	-
96-well culture plate	-	175 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	50 µl

* Maximum cuvette volume 200 µl