

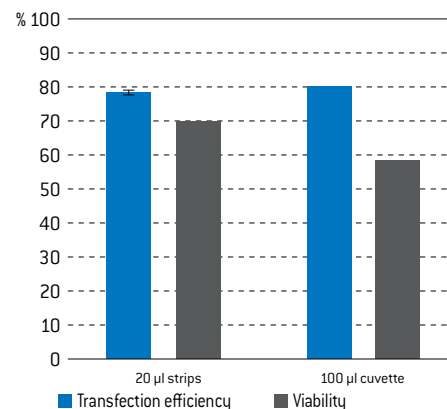
4D-Nucleofector™ Protocol for BA/F3 Cells

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

Mouse pro B cell; mostly single, round (some polymorph) cells in suspension (or occasionally in clumps)

Example for Nucleofection™ of BA/F3 Cells

Transfection efficiency and viability of BA/F3 cells 24 hours post Nucleofection™. BA/F3 cells were transfected with program CM-147 in 20 µl Nucleocuvette™ Strips (0.4 µg pmaxGFP™ Vector) or 100 µl Nucleocuvette™ Vessels (2 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) – 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% 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 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
- **Culture medium:** RPMI 1640 [Lonza; Cat. No. BE12-167F] supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, 2 mM Ultraglutamine 1 [Lonza; Cat. No. BE17-605E/U1, 10 ng/ml mouse IL-3 [BD; Cat. No. 354040]
- 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

- 1.1 Replace media every 2–3 days
- 1.2 Passage cells every 2–3 days. A subcultivation ratio of 1:15 to 1:20 is recommended. Use low spin centrifugation (90xg)
- 1.3 Cells should not be used for Nucleofection™ after passage number 25

Note

At high density (>2x10⁶ cells/ml), a cytokine-independent subclone may grow out relatively quickly

- 1.4 Seed out 1–3x10⁵ cells/ml in a T162 flask
- 1.5 Subculture 2–3 days before Nucleofection™

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 2] 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 2]
- 2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA [see Table 3]
- 2.7 Count an aliquot of the cells and determine cell density
- 2.8 Centrifuge the required number of cells [see Table 3] at 90xg for 10 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 the “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 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
- 2.18 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% 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

For more technical assistance, contact our Scientific Support Team:

USA /Canada

Phone: 800 521 0390 (toll-free)

Fax: 301 845 8338

E-mail: scientific.support@lonza.com

Europe and Rest of World

Phone: +49 221 99199 400

Fax: +49 221 99199 499

E-mail: scientific.support.eu@lonza.com

Lonza Cologne GmbH
50829 Cologne, Germany

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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		12-well plate	96-well plate
Culture medium	Pre-filled in plate	1000 µl	150 µl
	Added to sample post Nucleofection™	400 µl	80 µl
Volume of sample transferred to culture plate		complete sample (use supplied pipettes)	50 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		2 x 10 ⁵ (Lower or higher cell numbers may influence transfection results)	4 x 10 ⁵ (Lower or higher cell numbers may influence transfection results)
Substrate*	pmaxGFP™ Vector	2 µg	0.4 µg
	or plasmid DNA (in H ₂ O or TE)	1–5 µg	0.1–0.4 µg
or	siRNA	30–300 nM siRNA (3–30 pmol/sample)	30–300 nM siRNA (0.6–6 pmol/sample)
SG 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		CM-147	CM-147

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