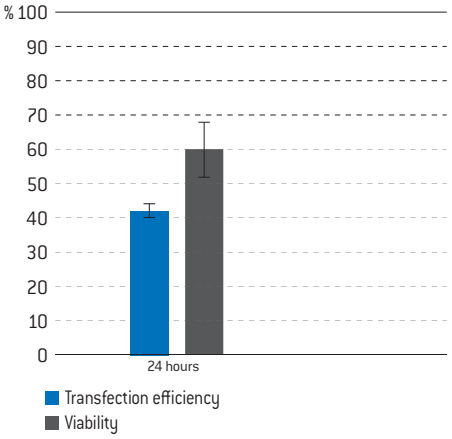
BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Protocol for Human Macrophages

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

Macrophages differentiated from human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood samples treated with an anticoagulant or from leukocyte-enriched buffy coat. Macrophages are large granular cells which adhere to plastic surfaces.



Example for Nucleofection™ of human macrophages

Transfection efficiency of human macrophages 24 hours post Nucleofection™. 1 x 10^5 cells were transfected with program DP-148 using 0.4 µg pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. Cells were analyzed 24 hours post Nucleofection™ using a FACSCalibur™ [Becton Dickinson]. Cell viability (CellTiterGlo® Viability Assay, Promega Cat. No.: G7570) is approximately 60 % after 24 hours.

Product Description

Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-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 Nucleovettes™ (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 Instruments, 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).

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- 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 dish for differentiation:** Poly-D-Lysine coated flasks (Becton Dickinson; Cat.No. 354537)
- **For detaching cells:** 0.5 mg/ml trypsin, 0.2 mg/ml EDTA in PBS
- **Differentiation medium:** RPMI 1640 (Lonza; Cat. No. 12-167F) supplemented with 10 % fetal calf serum (FCS), 100µg/ml streptomycin, 100 U/ml penicillin, and 2 mM glutamine, 1 % Na-pyruvate, 1 % NEAA (Non-Essential Amino Acids) and 50 ng/ml rHu M-CSF
- **Culture medium:** Macrophage-SFM (Invitrogen; Cat.No. 12065-074) supplemented with 10 % FCS and 2 mM glutamine
- PBS/BSA: PBS with 0.5 % BSA
- **For isolation:** Ficoll-Paque™ Plus (GE Healthcare; Cat. No. 17-1440-03)
- Prewarm appropriate volume of culture medium to 37 °C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Note
Transfection results may be donor-dependent.

Blood samples

1.1 Fresh human blood treated with an anticoagulant (e.g. heparin, citrate, ACD-A) or alternatively, leukocyte-enriched buffy coat not older than 8 hours

Preparation of PBMC

1.2 Pipet 15 ml Ficoll-Paque™ Plus in a 50 ml conical tube
1.3 Overlay Ficoll- Paque™ Plus with 35 ml blood sample and centrifuge at 750xg for 20 minutes at 20 °C in a swinging-bucket rotor without brake
1.4 Remove the upper layer leaving the mononuclear cell layer undisturbed at the interphase. Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube
1.5 Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4 °C. Remove the supernatant carefully
1.6 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4 °C. Remove the supernatant carefully
1.7 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 minutes at 4 °C. Remove the supernatant carefully
1.8 Resuspend cell pellet in 5 ml PBS/BSA and count the cells

Differentiation of macrophages

1.9 Plate 5 x 10^7–1 x 10^8 PBMC per 75cm^2 Poly-D-Lysine coated flask in differentiation medium
1.10 Enrich monocyte population by plastic adherence for 1–2 hours in an incubator at 37 °C in a 5 % CO_2 atmosphere
1.11 Discard supernatant with non-adherent cells and wash adherent monocytes 1 x with 15 ml prewarmed PBS per flask. Aspirate washing solution
1.12 Add 10 ml differentiation medium to each flask and differentiate monocytes for 7 days into macrophages
1.13 Replace media 2–3 x during the differentiation period

Trypsinization

1.14 Wash adherent macrophages once with PBS
1.15 Add Trypsin/EDTA solution (0.5 mg/ml trypsin and 0.2 mg/ml EDTA in PBS) to cover the cell monolayer (~3 ml per 75 cm^2 flask), and gently swirl the dish/flask to ensure an even distribution of the solution. Incubate the flask for 25–30 minutes at RT
1.16 Stop trypsinization by addition of supplemented RPMI without rHu M-CSF

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 adherent macrophages by trypsinization: (please see 1.14–1.16)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see table 3) at 200xg 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 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
2.19 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 % CO_2 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:

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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)	2 ml	180 µl
Cell number per Nucleofection™ Sample	5–7 x 10^5 (Lower or higher cell numbers may influence transfection results)	1 x 10^5 (Lower or higher cell numbers may influence transfection results)

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

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		5–7 x 10^5	1 x 10^5
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	2–5 µg	0.4–0.8 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ Solution		100 µl	20 µl
Program		DP-148	DP-148

* 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*
12-well culture plate	1.5 ml	-
96-well culture plate	-	100 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)	100 µl

* Maximum cuvette volume 200 µl