BioResearch

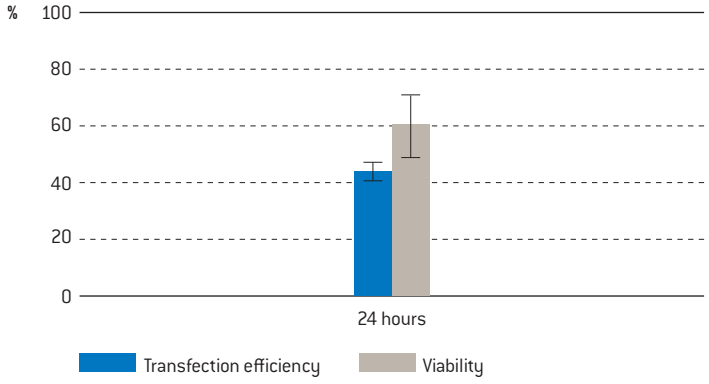
Lonza

Amaxa™ 96-well Shuttle™ Protocol for Human Macrophages

Cell Description

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 96-well Nucleofection™ of Human Macrophages



Transfection efficiency of human Macrophages 24 hours post Nucleofection™. 1×10^5 cells were transfected with program 96-DP-148 using 0.4 µg pmaxGFP™ Vector. Cells were analyzed 24 hours post Nucleofection™ using a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability (CellTiterGlo™ Viability Assay, Promega Cat. No.: G7570) is approximately 60 % after 24 hours.

Product Description

Recommended Kits

P3 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-3096
Size (reactions)	1×96
P3 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SP-3960
Size (reactions)	10×96
P3 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	10

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 expiry 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.

Notes

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-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.

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plates
- supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note
Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- Nucleocuvette™ compatible tips: 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
- 96-well culture plates or 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 for 25 minutes
- 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 with 0.5 % BSA (PBS/BSA)
- Ficoll-Paque™ Plus (GE Healthcare; Cat.No. 17-1440-03)
- Prewarm appropriate volume of culture media to 37°C (180 µl per sample)
- Appropriate number of cells (1×10^5 cells per sample; lower or higher cell numbers may influence transfection results)

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 750×g 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 350×g 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 160×g for 15 minutes at 4°C. Remove the supernatant carefully
1.7 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300×g 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 5×10^7–1×10^8 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× 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× 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™

One Nucleofection™ Sample Contains

- 1×10^5 cells
- 0.4–0.8 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 Primary Cell 96-well Nucleofector™ Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution

2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual "Nucleofector™ 96-well Shuttle™ System")

2.3 Select the appropriate Nucleofector™ Program **96-DP-148**

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 100 µl for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Pre-warm an aliquot of culture media to 37°C (80 µl per sample* see comments at the end of this chapter)

2.6 Prepare 0.4–0.8 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Harvest the adherent macrophages by trypsinization: (please see 1.14–1.16)

2.8 Take an aliquot of the cells and determine cell density

2.9 Centrifuge the required number of cells (1×10^5 cells per sample) at 200×g for 10 minutes at room temperature. Discard supernatant completely so that no residual medium covers the cell pellet

2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well 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.

2.11 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.12 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.13 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.14 After run completion, open retainer, carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.15 Resuspend cells with desired volume of pre-warmed media (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 80 µl of pre-warmed media*

2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 100 µl of resuspended cells to 100 µl pre-warmed media prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ results in most cases, however, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

3.1 Incubate the cells in a humidified 37°C/5 % CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours. If this is not the case, the incubation time can be prolonged up to 24 hours.

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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