BioResearch

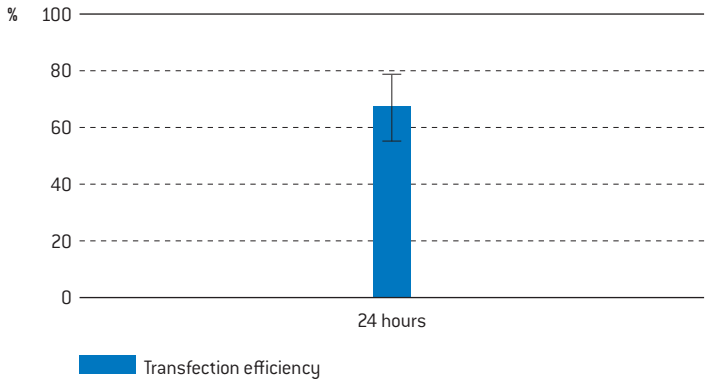
Lonza

Amaxa™ HT Nucleofector™ protocol for human monocytes

Cell description

The protocol is designed for primary human monocytes, freshly isolated from blood samples or buffy coats.

Example for Nucleofection™ of primary human monocytes



Average transfection efficiency of primary human monocytes 24 hours post Nucleofection™. Enriched human monocytes were transfected with program EA-100-AA and 0.4 µg of pmaxGFP™ vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability is usually around 75 % (% PI-negative monocytes) after 24 hours.

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kit

Cat. No.	V5SP-3002
Size (reactions)	2×384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-3010
Size (reactions)	10×384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well 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 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.

Notes

- This protocol only gives an outline for the handling and the Nucleofection™ of human monocytes. Please refer to more detailed preparation and cultivation protocols before starting the experiments.
- HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, 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.

- HT Nucleofector™ System
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well Nucleocuvette™ plate(s)
- 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, 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 medium: Please use your established human monocyte culture medium and required supplements or Clonetics™ Lymphocyte Growth Media-3 LGM-3™ for serum-free culture (Lonza, Cat. No. CC-3211) or BioWhittaker™ IMDM media for addition of 10 % serum (Lonza, Cat. No. BE12-722F)
- Prewarm appropriate volume of culture medium to 37°C (210 µl per sample)
- For enrichment: We recommend using the RosetteSep™ Isolation Kit for human monocytes (Stem Cell Technologies, Cat. No 15028). Alternatively, it is also possible to use the Monocyte Isolation Kit II (Miltenyi Biotec, Cat. No. 130-091-153) to purify the monocytes
- PBS/BSA for isolation: PBS containing 0.5 % BSA Ficoll-Paque™ (GE Healthcare; cat. No. 17-1440-03)
- Appropriate number of cells (1×10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note
This protocol only gives an outline for the handling and the Nucleofection™ of human monocytes. Experimental results and viability may vary within different blood samples or buffy coats.

Enrichment of monocytes from buffy coats

1.1 Centrifuge one buffy coat (≈ 60 ml) in two 50 ml tubes at 1200×g for 20 minutes at RT (brake off)
1.2 Remove most of the serum in the upper layer
1.3 Transfer the interphases (PBMC) together with traces of serum and erythrocytes (≈ 15 ml) into two fresh 50 ml tubes
1.4 Add 1000 µl cold Rosette-Cocktail (4°C) to each PBMC mix and vortex
1.5 Incubate 20 minutes at RT
1.6 Dilute 15 ml of the PBMC mix with 15 ml PBS/BSA and mix gently
1.7 Prepare two 50 ml tubes with 15 ml Ficoll-Paque™ and place 30 ml of the diluted PBMC-Mix as a layer on top of the Ficoll-Paque™
1.8 Centrifuge at 1200×g for 20 minutes at RT with brake off
1.9 Collect the interphase and transfer it to a fresh 50 ml tube on ice
1.10 Wash the enriched cells 2 x with ice-cold PBS/BSA
1.11 Resuspend cells in 5 ml PBS/BSA

Note
If you want to enrich monocytes from whole blood please refer to the RosetteSep™ Procedure for Human Monocyte Enrichment Cocktail (www.stemcell.com).

2. Nucleofection™

One Nucleofection™ sample contains

- 1×10^6 cells
- 0.2–1 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 HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution
2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for further details refer to the HT Nucleofector™ manuals)
2.3 Select the appropriate HT Nucleofector™ program EA-100-AA
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 170 µl* (see note at the end of this chapter) 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 medium to 37°C (40 µl per sample)
2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA(0.6–6 pmol/sample)
2.7 Count an aliquot of the cells and determine cell density
2.8 Centrifuge the required number of cells (1×10^6 cells per sample) at 90×g for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in 20 µl room temperature HT 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 384-well Nucleocuvette™ plates

B: Multiple substrates (e.g. library transfection)

– Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
– Add 2 µl substrates (maximum) to each well
– Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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.

2.10 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and sides of the wells without air bubbles.
2.11 Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
2.12 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position
2.13 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for further details refer to the HT Nucleofector™ manuals)
2.14 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel
2.15 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media*
2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 30 µl of resuspended cells to 170 µl pre-warmed media

***Note**

The indicated cell numbers and volumes have been found to produce optimal 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.

Additional information

Up-to-date List of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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