BioResearch

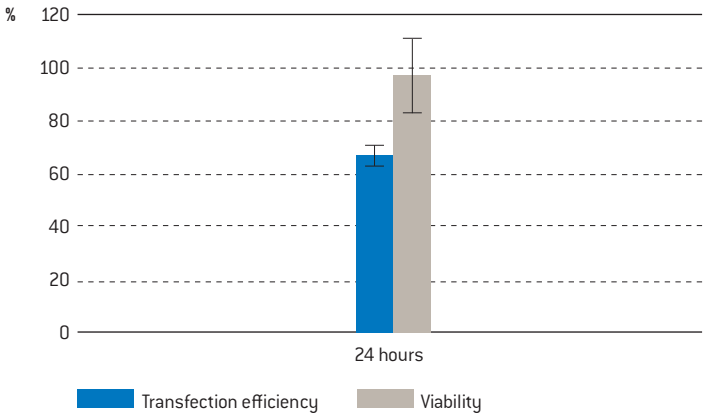
Lonza

Amaxa™ HT Nucleofector™ protocol for human stem cells (H9)

Cell description

This protocol is designed for human stem cell line H9. Pluripotent stem cells, adherent.

Example for Nucleofection™ of human stem cell line H9



Transfection efficiency of human embryonic cells (H9) cells 24 hours post Nucleofection™. $2x10^5$ cells were transfected with program CB-150-AA using 0.8 µg pmaxGFP™ vector. Cells were analyzed 24 hours post Nucleofection™ by FACS with HTS option. Cell viability was analyzed by PI staining. Cell counts were compared to non transfected control (data by courtesy of Jennifer Moore, Rutgers University, Piscataway, USA).

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2x384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-3010
Size (reactions)	10x384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 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 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

- This 384-well Nucleofector™ kit is based upon data obtained from external co-operation partners experienced in working with human stem cells. The H9 cells used were grown without feeder cells on BD Matrigel™ (BD Biosciences). The protocol may work on H9 cultured by other methods, but we strongly recommend contacting our scientific support team for further information before starting experiments.
- HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, the 4D-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™ 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
- 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 other culture plates coated with BD Matrigel™ (BD Biosciences)
- For detachment of cells: Use Accutase (PAA)
- Culture medium: mTeSR™ medium (StemCell Technologies; Cat. No. #05850)
- Pre-warm appropriate volume of culture medium at 37°C (192 µl per sample)
- Appropriate number of cells ($2x10^5$ to $4x10^5$ cells per sample)

1. Pre Nucleofection™

Note
Transfection results may vary due to different culture conditions prior and post Nucleofection™.

Important considerations

Single cell suspension

- We recommend transfecting the cells in a single cell suspension. Nucleofection™ of clumps lead to lower transfection efficiency and less reproducibility (for details see reference 1)
- If single cell suspension passage is not established, please do some pre-experiments by testing Accutase (reference 2) and Trypsin (reference 3) for detachment. Cultivate the cells afterwards and analyze which method led to highest viability and lowest differentiation
- The use of apoptosis inhibitors like ROCK inhibitor (reference 4) and neurotrophins (reference 1) have been reported to increase viability of hES cells. The results presented herein were gained without the use of these molecules. However, depending on hESC culture conditions it might be advantageous to use ROCK inhibitor or neurotrophins to obtain higher viabilities
- We recommend using the cell culture method described in this optimized protocol. However, Nucleofection™ conditions described in this protocol may work on H9 cells cultured on gelatine coated plates and feeder cells. For further information please contact our scientific support team

Cell culture recommendations

1.1 Replace media every day

1.2 Cells should be passaged 1 time per week with a sub cultivation ratio of 1 : 3 to 1 : 10. You may use Collagenase, Dispase or another enzyme for this purpose

1.3 If your H9 cells are usually cultured on feeder cells, passage them once to BD Matrigel™ (BD Biosciences) coated plates to remove the feeder cells (described in reference 2)

1.4 Prior to Nucleofection™ detach the hES cells from the BD Matrigel™ (BD Biosciences) plates by incubation with Accutase for 5 minutes at 37°C. Dissociate the cells into a single cell suspension by pipetting the suspension carefully up and down 4–6 times. Add medium to stop Accutase

2. Nucleofection™

One Nucleofection™ sample contains

- $2x10^5$ cells
- 0.4–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4–0.8 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 primary cell HT Nucleofector™ solution

Note

Human stem cells are quite sensitive to environmental conditions. Therefore please ensure you proceed with the Nucleofection™ steps as fast as possible.

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 details refer to the HT Nucleofector™ manuals)

2.3 Select the appropriate HT Nucleofector™ program **CB-150-AA**

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 152 µl* (see note at the end of this chapter) for one well of a 96-well plate coated with BD Matrigel™ (BD Biosciences) and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Equilibrate an aliquot of culture media to 37°C (40 µl* per sample) in an incubator

2.6 Prepare 0.4–1 µg plasmid DNA or 0.4–0.8 µg pmaxGFP™ vector. For siRNA experiments we recommend starting with 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Harvest the cells using trypsin or accutase (please see 1.4)

2.8 Count an aliquot of the detached cells and determine cell density

2.9 Centrifuge the required number of cells ($2x10^5$ cells per sample) at 115xg for 3 minutes at room temperature

2.10 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.

2.11 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. 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 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 384-well plates: Transfer 46 µl of resuspended cells to 152 µl pre-warmed media prepared in 96-well culture plates coated with BD Matrigel™ (BD Biosciences) (plates coated with gelatine and pre-plated inactivated feeder cells may work as well, but possibly lead to lower performance)*

2.17 If post Nucleofection™ cell culture is done in BD Matrigel™ (BD Biosciences) coated 96-well plates, centrifuge the culture plates loaded with cells at this point to guarantee proper attachment of the cells (70xg, 3 minutes, room temperature)

Notes

- The BD Matrigel™ (BD Biosciences) plates used for culturing of hES should be fresh. Storage of BD Matrigel™ plates for more than 7 days led to reduced attachment of the cells post Nucleofection™.
- * 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

3.2 As cells were plated at high density post Nucleofection™, a passage step 48 hours post Nucleofection™ might be necessary

Additional information

Up-to-date list of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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References

1. Kristi A. Hohenstein et al. (2008) Nucleofection™ Mediates High-efficiency Stable gene Knockdown and Transgene Expression in Human Embryonic Stem Cells. Stem Cells First published online March 20, 2008; doi:doi:10.1634/stemcells.2007-0857
2. Henrike Siemen et al. (2005). Nucleofection™ of Human Embryonic Stem Cells. Stem Cells and Development 14: 378-383
3. Catharina Ellerström et al. (2007). Facilitated expansion of human embryonic stem cells by single cell enzymatic dissociation. Stem Cells 25:1690-1696
4. Kiichi Watanabe et al. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nature Biotechnology 25 (6): 681-686

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