BioResearch

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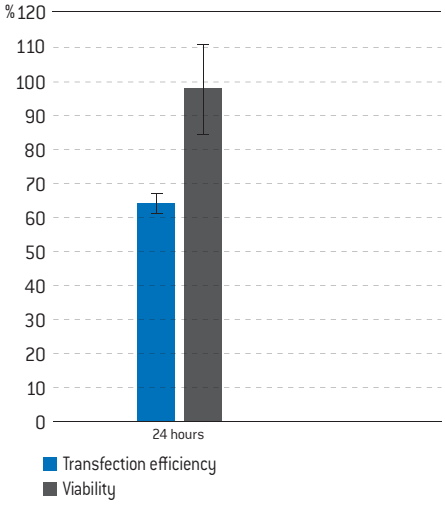
Amaxa® 4D-Nucleofector® Protocol for Human Stem Cell Line H9

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

Pluripotent stem cells, adherent

This protocol 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.
For Nucleofection® of other human stem cell lines please refer to the "Amaxa® 4D-Nucleofector® Basic Protocol for Human Stem Cells".



Example for Nucleofection® of Human Stem Cell Line H9

Transfection efficiency of human embryonic cells (H9) 24 hours post Nucleofection®. 2 x 10^5 cells were transfected with program CB-150 using 0.8 µg pmaxGFP® Vector in 20 µl Nucleocuvette® Strips. Cells were analyzed 24 hours post Nucleofection® by FACS. 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 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 (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg	30 µg	30 µg
pmaxGFP® Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	-	-	45 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 could be only 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
- 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 or other culture plates coated with BD Matrigel™ (BD Biosciences)
- **For detaching cells:** Use Accutase (PAA Laboratories, Cat. No. L11-007)
- **Culture medium**: mTeSR® medium (StemCell Technologies; Cat. No. #05850)
- 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 vary due to different culture conditions prior and post Nucleofection®.

Important considerations - Single cell suspension:

1. 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)
2. 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
3. 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
4. We recommend using the cell culture method described in this optimized protocol. However, Nucleofection® Conditions described in this protocol may also 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®

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 cells using trypsin or accutase (please see 1.4)

2.8 Count an aliquot of the cells and determine cell density

2.9 Centrifuge the required number of cells (see table 3) at 115xg for 3 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 table5).
2.20 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)
2.21 Note 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®

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

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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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: 378-3832.
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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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)	1 ml	200 µl
Cell number per Nucleofection® Sample	8×10^5 (Lower or higher cell numbers may influence transfection results)	$2 \times 10^5 – 4 \times 10^5$ (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		8×10^5	$2 \times 10^5 – 4 \times 10^5$
Substrate*	**pmaxGFP® Vector**	2 µg	0.4 – 0.8 µg
or	**plasmid DNA (in H_2O or TE)**	1 – 5 µg	0.4 – 1 µg
or	**siRNA**	30 – 300nM siRNA (3 – 30 pmol/sample)	30 – 300nM siRNA (0.6 – 6 pmol/sample)
P3 Primary Cell 4D-Nucleofector® X Solution		100 µl	20 µl
Program		CB-150	CB-150

* 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*
24-well culture plate	500 µl	-
96-well culture plate	-	120 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)	50 µl

* Maximum cuvette volume 200 µl