BioResearch

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Amaxa™ 96-well Shuttle™ Basic Protocol for Human Stem Cells

Cell Description

For Human Stem Cells; e.g. H1, H7, H14, HS306 ; pluripotent cells, adherent.

Note

This basic protocol describes how to easily define optimal Nucleofection™ Conditions for different human stem cells (e.g. H1, H7, H14, HS306). We recommend first testing a set of pre-selected Nucleofector™ Programs together with two of our Primary Cell 96-well Nucleofector™ Kits:

- P3 Primary Cell 96-well Nucleofector™ Kit
- P4 Primary Cell 96-well Nucleofector™ Kit

For subsequent experiments simply use the kit which yields the best results.

If you have questions regarding your human stem cell of interest, please contact our Scientific Support Team for further help with the optimization.

Product Description

Recommended Kits

- P3 Primary Cell 96-well Nucleofector™ Kits
- P4 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 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 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	10

Cat. No.	V4SP-4096
Size (reactions)	1×96
P4 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-4960
Size (reactions)	10×96
P4 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 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.

Optimization Guidelines

The initial optimization experiment is comprised of 32 reactions, using 2 Nucleocuvette™ Modules: 7 different Nucleofector™ Programs are tested in duplicate with 2 Nucleofector™ Solutions plus 1 control. The program and 96-well Nucleofector™ Solution which turned out to be the most appropriate Nucleofection™ Condition should be used for all subsequent transfections.

	P3 Primary Cell Nucleofector™ Solution		P4 Primary Cell Nucleofector™ Solution	
	1	2	3	4
A	96-CA-137	96-CA-137	96-CA-137	96-CA-137
B	96-CB-150	96-CB-150	96-CB-150	96-CB-150
C	96-CD-118	96-CD-118	96-CD-118	96-CD-118
D	96-CE-118	96-CE-118	96-CE-118	96-CE-118
E	96-CM-113	96-CM-113	96-CM-113	96-CM-113
F	96-DC-100	96-DC-100	96-DC-100	96-DC-100
G	96-DN-100	96-DN-100	96-DN-100	96-DN-100
H	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)

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 prior to Nucleofection™
- 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) or Matrix TallTips™ (Matrix Technologies Corp., Cat. No. 7281). 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
- For detaching the cells: Accutase solution (PAA Laboratories, Cat. No. L11-007) or 0.05 or 0.25 % Trypsin/EDTA solution (Invitrogen, Cat. No. 253000-54 or 252000-56)
- Appropriate volume of culture medium
- Appropriate number of cells (2×10^5 to 4×10^5 cells per sample)

For Culture with Feeder Cells:

- Prewarm appropriate volume of culture media at 37°C (200 µl per sample); DMEM:F-12 (Lonza, Cat. No. 12-719F) supplemented with 15–20 % serum replacement (Invitrogen, Cat. No. 10828-028), 1–2 % nonessential amino acids (Lonza, Cat. No. 13-114E), 1–4 mM L-glutamine (Lonza, Cat. No. 17-605C), 0.1 mM 2-Mercaptoethanol (Invitrogen, Cat. No. 21985-023) and 4–8 ng/ml fibroblast growth factor-2 (Milipore, Cat. No. GF003AF-MG)
- Prepare a 96-well plate coated with gelatine and inactivated feeder cells (one well per sample) 24 hours before Nucleofection™

For Feeder-Free Culture:

- Prewarm appropriate volume of culture media at 37°C (200 µl per sample; mTesSR™ 1 medium (StemCell Technologies, Cat. No. 05850)
- Prepare a 96-well culture plate coated with Matrigel™ (BD Biosciences, Cat. No. 354277)

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 single cell suspension. Nucleofection™ of clumps leads 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 3) and Trypsin (reference 4) 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 5) and neurotrophins (reference 6) have ––been reported to increase viability of hES cells. Depending on hESC culture conditions, it might be advantageous to use ROCK inhibitor or neurotrophins to obtain higher viabilities

Cell Culture Recommendations

1.1 Replace media every day

1.2 Cells should be passaged 1–2 times per week with a sub-cultivation ratio of 1 : 3 to 1 : 10. You may use Collagenase, Dispase or another enzyme for this purpose

Detachment of Stem Cells

1.3 **A. Harvest of stem cells cultured on feeder cells**

There are three possibilities to remove feeder cells from your stem cell culture prior to Nucleofection™:

- If your stem cells are usually cultured on feeder cells, passage them once to Matrigel™ coated plates to remove the feeder cells (described in reference 2). Then proceed to step 1.3 B
- Cultivate the cells on feeder cells until the day of the experiment. Detach the stem cells with Collagenase. Dissociate the clumps with Accutase into a single cell suspension
- Cultivate the cells on feeder cells until the day of the experiment. Detach all cells with Accutase. Incubate the cells on an uncoated cell culture flask for 1 hour in a humidified 37°C/5 % CO_2 incubator.
- The feeder cells will attach and the stem cells will stay in suspension. Harvest the cells in suspension

B. Harvest of feeder-free stem cell cultures

- Prior to Nucleofection™ detach the hES cells from the Matrigel™ 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

- 2×10^5 cells
- 0.4–1µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4–0.8 µg pmaxGFP™ Vector
- 20 µl 96-well 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 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 appropriate Nucleofector™ Program. Please try all 7 Nucleofector™ Programs (**96-CA-137, 96-CB-150, 96-CD-118, 96-CE-118, 96-CM-113, 96-DC-100** and **96-DN-100**) initially with both Nucleofector™ Solutions to determine the most appropriate Nucleofection™ Condition for your specific stem cell type

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 120 µl (see comments at the end of this chapter) for one well of a 96-well plate coated with Matrigel™ or gelantine and feeder cells and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Pre-warm /equilibrate an aliquot of culture media to 37°C (80 µl per sample)

2.6 Prepare 0.4–1 µg plasmid DNA or 0.4–0.8 µg pmaxGFP™ Vector

2.7 Harvest the cells (please see 1.3)

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

2.9 Centrifuge the required number of cells (2×10^5 cells per sample) at 115×g for 3 minutes at room temperature

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. 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. Make sure the sample covers the bottom of the well, if necessary gently tap the Nucleocuvette™ Plate. Avoid air bubbles while pipetting.

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

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

2.13 After retainer opening, carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.14 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

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 80 µl of resuspended cells to 120 µl pre-warmed media prepared in 96-well culture plates coated with Matrigel™ or gelantine and feeder cells

2.16 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 (70×g, 3 minutes, room temperature)

Notes

The Matrigel™ plates used for culturing of human stem cells should be fresh. Storage of these plates for more than 7 days leads to reduced attachment of the cells post Nucleofection™

The indicated plating cell numbers and volumes produce optimal 96-well Nucleofection™ Results in most cases. However, you may wish to test an extended range of cell numbers depending on your specific needs.

3. Post Nucleofection™

3.1 Incubate the cells in a humidified 37°C/5 % CO_2 incubator until analysis. Gene expression 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™ using Collagenase or Dispase might be necessary

Note

The plating density post Nucleofection™ is a critical aspect for the viability of human stem cells. Our experience is that higher densities lead to better viability of the cells. Therefore we recommend plating human stem cells at densities from 4×10^5 to 6.5×10^5 cells per cm^2.

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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References

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