Human Stem Cell Nucleofector® Kit 1 and 2

For Human Stem Cells

e.g. BG01V, H1, H7, H9.2, HSF6, RH1 and RH6

Note **In order to use this kit, you should already have determined the optimal combination of program and Nucleofector® Solution for your cells using the Human Stem Cell Nucleofector® Starter Kit [Cat.No. VPH-5002]. If the Human Stem Cell Nucleofector® Solution 1 yields the best results, simply use the Human Stem Cell Nucleofector® Kit 1 [Cat. No. VPH-5012] or the Human Stem Cell Nucleofector® Kit 2 [Cat. No. VPH-5022] if Human Stem Cell Nucleofector® Solution 2 yields the best results.**

Product Description

Human Stem Cell Nucleofector®	Kit 1	Kit 2
Cat. No.	VPH-5012	VPH-5022
Size (reactions)	25	25
Human Stem Cell Nucleofector® Solution 1or 2	2.25 ml	2.25 ml (2.05 ml + 10% overfill)
Supplement 1	0.5 ml	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg	30 µg
Certified cuvettes	25	25
Plastic pipettes	25	25
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.	

Required Material

Note **Please make sure that the entire supplement is added to the Nucleofector® Solutions. The ratio of Nucleofector® Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector® Device
- Supplemented Nucleofector® Solutions at room temperature prior to Nucleofection®
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- Culture dishes of your choice
- **For detaching cells:** Accutase solution [PAA Laboratories, Cat. No. L11-007] or 0.05 or 0.25% Trypsin/EDTA solution
- **Medium for culture with feeder cells:** DMEM F-12 [Lonza, Cat.No. 12-719F] supplemented with 15 – 20% Knockout™ 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 and 4 – 8 ng / ml fibroblast growth factor-2 [Milipore, Cat. No. GF003AF-MG]
- **Plates for culture with feeder cells:** Prepare a 24-well plate coated with gelatine and inactivated feeder cells (one well per sample) 24 hours before Nucleofection®
- **Medium for feeder-free culture:** mTesSR™ 1 medium [StemCell Technologies, Cat. No. 05850]
- **Plates for feeder-free culture:** Prepare a 24-well culture plate coated with BD Matrigel™ [BD Biosciences, Cat. No. 354277]
- Prewarm appropriate volume of culture medium to 37°C (1 ml per sample)
- Appropriate number of cells (8 x 10^5 cells per sample; lower or higher cell numbers may influence transfection results)

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 enzymes for this purpose

Detachment of stem cells

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 BD Matrigel™ coated plates to remove the feeder cells (described in reference 2). Then proceed to step 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 BD 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

8 x 10^5 cells
1 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® or 30 – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Human Stem Cell Nucleofector® Solution 1 or 2

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 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 500 ml* (see comments at the end of this chapter) for one well of a 24-well plate coated with BD Matrigel™ or gelatine and feeder cells and pre-incubate/equilibrate plates in a humidified 37°C/5%
2.3 Harvest the cells (see chapter 1)
2.4 Count an aliquot of the detached cells and determine cell density
2.5 Centrifuge the required number of cells (8 x 10^5 cells per sample) at 115xg for 3 minutes at room temperature
2.6 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
2.7 Combine 100 µl of cell suspension with **1 – 5 µg DNA**, 2 µg pmaxGFP® (recommended for initial optimization) or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates
2.8 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
2.9 Select the appropriate Nucleofector® Program initially determined using the Nucleofector® Starter Kit: **A-012**, **A-013**, **A-023**, **A-027** or **B-016** (A-12, A-13, A-23, A-27 or B-16 for Nucleofector® I Device)

2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.11 Take the cuvette out of the holder once the program is finished
2.12 Add ~500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the 24-well plate (final volume 1 ml media per well/sample) coated with BD Matrigel™ or gelatine and feeder cells. Use the supplied pipettes and avoid repeated aspiration of the sample

Note **The BD Matrigel™ coated 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®.**

*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 and DNA amounts.**

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® 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 x 10^5 to 6.5 x 10^5 cells per cm^2.**

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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