Lonza

Amaxa™ Basic Nucleofector™ Kit for Primary Mammalian Smooth Muscle Cells

For Primary Mammalian Smooth Muscle Cells (SMC)

Cells derived from mammalian smooth muscle cell tissues from various organs; adherent long tapering cells

Note **Mammalian smooth muscle cells display significant phenotypic variations due to the wide range of both species and body sites from which they may be sourced. You can determine the optimal Nucleofection™ condition for your smooth muscle cells using the Basic Nucleofector™ Kit for Primary Mammalian Smooth Muscle Cells [Cat. No. VPI-1004]. Please find some guidelines on smooth muscle cell culture for Nucleofection™ and on the transfection procedure using our Basic Nucleofector™ Kit below. However, we recommend referring to more detailed culture protocols before you start the experiments. Having tested various smooth muscle cell types, high transfection efficiencies could be achieved using one of the programs indicated below. If you do not attain satisfying results with your smooth muscle cells of interest, please contact our Scientific Support Team for further help with the optimization. On our website (www.lonzabio.com) we provide a form you might use to enter the results achieved with the Basic Kit.**

Product Description

Cat. No.	**VPI-1004**
Size (Reactions)	25
Basic Nucleofector™ Solution for Mammalian SMC	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP™ vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	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.

Optimization Guidelines

The initial optimization experiment is comprised of 6 reactions: 5 different Nucleofector™ Programs are tested with 1 Nucleofector™ Solution plus 1 control (no program). The Nucleofector™ Program which turns out to be the most appropriate should be used for all subsequent transfections. A further fine tuning of the Nucleofection™ condition can be performed with the help of our Scientific Support Team.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Program	A-033	D-033	P-013	P-024	U-25	No program

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. 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™ Solution at room temperature
- 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 6-well culture dish (3 wells/sample) or culture system of your choice
- **For trypsinization:** Reagent Pack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) [Lonza, Cat. No. CC-5034]. Alternatively if cells hardly detach: Trypsin 0.5%-EDTA 0.2%
- **Culture medium:** Please use a medium especially suited for the culture of primary smooth muscle cells, e.g. SmGM®-2 BulletKit®, [Lonza, Cat. No. CC-3182], alternatively, DMEM supplemented with 10% FCS or a different special medium recommended for your epithelial cell type containing all required supplements. **We recommend storing 40 ml aliquots of the prepared medium at -80°C. Do not use medium stored at 4°C for more than two days, as this may lead to reduced cell viability and transfection efficiency**
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (0.5–1 x 10^6 cells per sample)
  Minimal cell number: 2 x 10^5 cells (a lower cell number may lead to a major increase in cell mortality)
  Maximum cell number: 2 x 10^6 cells

1. Pre Nucleofection™

Note **Transfection results may be donor-dependent.**
Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.

Cell culture recommendations

1.1 Replace medium every 2 days
1.2 Cells should be passaged after reaching 80% confluency
1.3 Do not use cells after passage 15 for Nucleofection™
1.4 Optimal confluency for Nucleofection™ 80%; higher confluency may reduce viability

Trypsinization

1.5 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
1.6 For harvesting, incubate the cells up to 10 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.7 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

2. Nucleofection™

One Nucleofection™ Sample contains

0.5–1 x 10^6 cells
1–5 µg plasmid DNA (in 1–5 µl H_2O or TE) or 2 µg pmaxGFP™ vector or 30–300 nM siRNA (3–30 pmol/sample)
100 µl Nucleofector™ Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Prepare 6-well plates (3 wells/sample) by filling appropriate number of wells with 1 ml/well of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 For rinsing the cuvette pre-warm in an Eppendorf tube 500 µl supplemented culture medium per sample at 37°C
2.4 Harvest the cells by trypsinization (please see 1.5–1.7)
2.5 Count an aliquot of the trypsinized cells and determine cell density
2.6 Centrifuge the required number of cells (0.5–1 x 10^6 cells per sample) at 100xg for 10 minutes at room temperature
2.7 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector™ Solution per sample
2.8 Combine 100 µl of cell suspension with **1–5 µg DNA**, 2 µg pmaxGFP™ vector (recommended for initial optimization), or **30 nM–300 nM siRNA** (3–30 pmol/sample) or other substrates
2.9 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
2.10 Select the appropriate Nucleofector™ Program. Please try all 5 Nucleofector™ Programs initially to determine the most appropriate one for your smooth muscle cell type for all subsequent experiments **A-033, D-033, P-013, P-024 or U-025** (A-33, D-33, P-13, P-24 and U-25 for Nucleofector™ I Device)
2.11 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program
2.12 Take the cuvette out of the holder once the program is finished
2.13 Add immediately ~500 µl of the pre-warmed culture media to the cuvette and **gently** transfer the sample into the Eppendorf tube. Use the supplied pipettes and avoid repeated aspiration of the sample
2.14 Transfer the samples from the Eppendorf tubes into the prepared 6-well plates. Seed one sample in 3 wells of one 6-well plate

3. Post Nucleofection™

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis and change medium 16–18 hours post Nucleofection™
3.2 Gene expression or down regulation, respectively, is often detectable after only 4–8 hours but ideally, cells should be left undisturbed until medium change

Additional Information

For an up-to-date list of all primary smooth muscle cells successfully transfected with this Basic Nucleofector™ Kit, please refer to:
www.lonza.com/cell-database

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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