Amaxa™ Basic Nucleofector™ Kit for Primary Mammalian Glial Cells

For Primary Mammalian Glial Cells

Macroglia cells derived from mammalian central and peripheral nervous system; adherent cells

Note **Mammalian glial 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 glial cells using the Basic Nucleofector™ Kit for Primary Mammalian Glial Cells [Cat. No. VPI-1006]. Please find some guidelines on glial 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 glial cell types, high transfection efficiencies could be achieved using one of the programs indicated below. If you do not attain satisfying results with your glial 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 Cell Line Optimization Kit**

Product Description

Cat. No.	**VPI-1006**
Size (reactions)	25
Basic Nucleofector™ Solution for Mammalian Glial Cells	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	E-013	G-013	O-017	T-020	No program

The Basic Nucleofector™ Kit for Primary Mammalian Glial Cells has been tested successfully for the following cell types:

	Optimal Nucleofector™ Program	Optimal cell number per reaction	Transfection efficiency	Viability
Mouse Astrocytes	T-020	2 x 10^6	60%	60-70%
Rat Astrocytes	T-020	2 x 10^6	59%	70-80%
Rat Oligodendrocytes	O-017	5 x 10^6	44%	60%

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
- Culture dishes (Ø 6 cm/sample) or culture system of your choice. Depending on the cell type, coat culture dishes or coverslips, e.g. with poly-ornithine or with poly-D-lysin
- **For trypsinization:** Please use trypsin as recommended by the cell supplier e.g. 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 use 0.5 mg/ml Trypsin, 0.2 mg/ml EDTA in PBS and supplemented culture media
- **Culture medium:** Please use a medium especially suited for the culture of primary glial cells, e.g. Astrocyte Cell Medium, AGM™ with BulletKit™ Additives [Lonza, Cat. No. CC-3186] or a different special medium recommended for your glial cell type containing all required supplements
- Prewarm appropriate volume of culture medium to 37°C (5.5 ml per sample)
- **Appropriate number of cells**: 2–3 x 10^6 cells per sample. Minimal cell number: 1 x 10^6 cells (a lower cell number may lead to a major increase in cell mortality). Maximum cell number: 5 x 10^6

1. Pre Nucleofection™

Note **Transfection results may be source- and species-dependent. Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations. For Nucleofection™ of rat and mouse astrocytes as well as rat oligodendrocytes you will find optimized protocols in our online cell database: www.lonza.com/celldatabase. These protocols include also cell culture recommendations and isolation protocols for the different cell types.**

Cell culture recommendations

1.1 Replace 50% of the media every 3–5 days with warm, fresh supplemented medium
1.2 Avoid repeated warming and cooling of the medium
1.3 Astrocytes can be trypsinized and re-plated when the cells are confluent
1.4 For subcultivation plate 4000 cells /cm^2

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 10–15 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

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

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Prepare 6 cm dishes (if necessary pre-coated with e.g. poly-ornithine or poly D-lysin) by filling appropriate number of dishes with 5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Harvest the cells by trypsinization (please see 1.5–1.7)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (2–3 x 10^6 cells per sample) at 100xg for 10 minutes at room temperature. Remove supernatant completely
2.6 Resuspend the cell pellet carefully in 100 µl room-temperature Nucleofector™ Solution per sample

Note **Avoid leaving the cells in Nucleofector™ Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability and gene transfer efficiency**

2.7 Combine 100 µl of cell suspension with 1–5 µg DNA, 2 µg pmaxGFP™ vector 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. Please try all 5 Nucleofector™ Programs initially to determine the most appropriate one for your glial cell type for all subsequent experiments: **A-033, E-013, G-013, O-017** and **T-020** (**A-33, E-13, G-13, O-17** and **T-20** for Nucleofector™ I Device)

2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program by pressing the X-button
2.11 Take the cuvette out of the holder once the program is finished
2.12 Immediately add ~ 500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6 cm culture dish (final volume 5.5 ml media per dish) or culture dish with the coated coverslip. Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 Gene expression or down regulation, respectively, is often detectable after only 4–8 hours but ideally, cells should be left undisturbed for 24 hours

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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