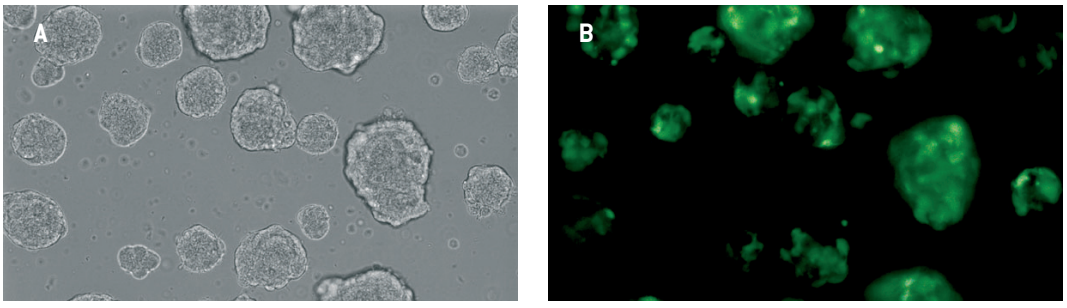
# Amaxa® Mouse NSC Nucleofector® Kit

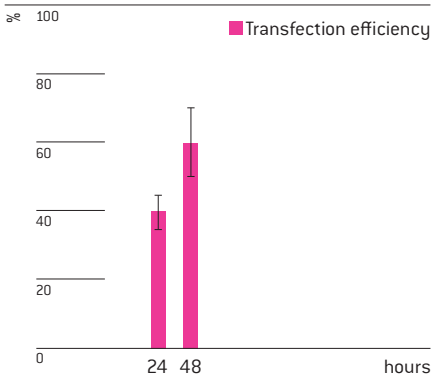
## For Mouse Neural Stem Cells (NSC)

**Note** This protocol is optimized for primary neural stem cells isolated from mouse embryos (E14-15) and grown as neurospheres. For cells grown adherently, different settings may be required for successful Nucleofection®.

### Example for Nucleofection® of primary mouse NSC




**Primary mouse neural stem cells isolated from the lateral ventrical wall were transfected with the Mouse NSC Nucleofector® Kit, Program A-033 and a plasmid encoding the enhanced green fluorescent protein eGFP.** Neurospheres were analyzed 48 hours post Nucleofection® using light (A) and fluorescence microscopy (B). Photograph courtesy by Dr. L.Wikstrom et al., NeuroNova AB, Stockholm, Sweden.




**Average transfection efficiency of primary mouse NSC.** Cells were transfected with program A-033 and 5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 24 and 48 hours post Nucleofection® by fluorescence microscopy. Cell viability is usually around 85-90%.

## Product Description

| | |
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| Cat. No. | **VPG-1004** |
| Size (reactions) | 25 |
| Cell Line Nucleofector® Solution V | 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. |

## 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
- 12-well culture dish or culture system of your choice
- **Culture medium I**: 1 : 1 mixture of DMEM and F12 supplemented with 0.6% glucose, 2 mM glutamine, 5 mM HEPES, 3 mM $NaHCO_3$, N2 supplement (1 : 100) [Invitrogen; Cat.No. 17502-048], 10 ng/ml EGF [Sigma; Cat.No. E1257] and 10 ng/ml bFGF [Sigma; Cat.No.F5392]
- **Culture medium II**: Culture medium I without EFG and bFGF, but supplemented with 1% FCS
- Prewarm appropriate volume of culture medium I to 37°C (0.8 ml per sample)
- Appropriate number of cells ($4 \times 10^6 - 5 \times 10^6$ cells per sample; minimal cell number: $1 \times 10^6$ cells, a lower cell number may lead to a major increase in cell mortality; maximum cell number: $6 \times 10^6$)

## 1. Pre Nucleofection®

### Isolation/preparation of cells

**Note** **This protocol only gives an outline for the isolation and culture of primary mouse NSC. Please refer to more detailed protocols in the literature (see chapter "Additional Information") before starting the experiments.**

1.1 Separate heads from mouse embryos (E14-15) for Nucleofection®
1.2 Dissect brains and extract cortices, midbrain and striatum
1.3 Alternatively, extract spinal cords
1.4 Transfer tissue into a Petri dish with culture medium I
1.5 Mechanically dissociate cells with a fire-polished Pasteur pipette
1.6 until the suspension is homogenous
1.7 Count cell numbers
1.8 Centrifuge for 5 minutes at 80xg
1.9 Suspend cells in culture medium I and seed at the following densities:
~4,000 cells / 24 well plate
~50,000 cells / 6 cm dish
~100,000 cells / 10 cm dish
1.10 Change medium every other day and passage the cells weekly
1.11 Use cells passaged 3 – 4 times for Nucleofection®
1.12 Spin cells down for 5 minutes at 80xg
1.13 Carefully resuspend cells in culture medium I to get a single cell suspension
1.14 Count cell numbers
1.15 Spin down the required number of cells ($4 - 5 \times 10^6$ cells per Nucleofection® Sample) at 80xg for 5 minutes
1.16 Remove supernatant completely

## 2. Nucleofection®

| One Nucleofection® Sample contains |
|---|
| 4 – 5 x $10^6$ cells |
| 2 – 10 µg plasmid DNA (in 1 – 5 µl $H_2O$ or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample) |
| 100 µl Mouse Neural Stem Cell Nucleofector® Solution |

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 12-well plates by filling appropriate number of wells with 0.3 ml of culture medium I and pre-incubate/equilibrate plates in a humidified 37°C/5% $CO_2$ incubator
2.3 Resuspend the cell pellet carefully in room-temperature Mouse NSC Nucleofector® Solution to a final concentration of 4 – 5 x $10^6$ cells/100 µl

Note **Avoid leaving the cells in Nucleofector® Solution for extended periods of time (longer than 30 minutes), as this may reduce cell viability and gene transfer efficiency.**

2.4 Combine 100 µl of cell suspension with **2 – 10 µg DNA**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM siRNA** (3 – 30 pmol/sample) or other substrates
2.5 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.6 Select the appropriate Nucleofector® Program **A-033** (or **A-33** for Nucleofector® I Device)
2.7 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.8 Take the cuvette out of the holder once the program is finished
2.9 Immediately add ~500 µl of the pre-equilibrated culture medium I to the cuvette and gently transfer the sample into the prepared 12-well plate (final volume 0.8 ml media per well). 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
3.2 Replace culture medium I after 24 hours with either culture medium I (if cells shall be grown further as undifferentiated neurospheres) or culture medium II (if cells shall be differentiated into neurons or astrocytes)
3.3 Cells can be grown in culture for at least one week. Change medium every other day
3.4 Depending on the transferred gene, expression is often detectable after 6 – 8 hours and lasts up to 6 – 8 days

## 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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### Additional References

1. Pressmar et al. [2001] Invest. Ophthalmol. Vis. Sci. 42: 3311-3319.
2. Kalyani et al. [1997] Dev. Biol. 186: 202-223.

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