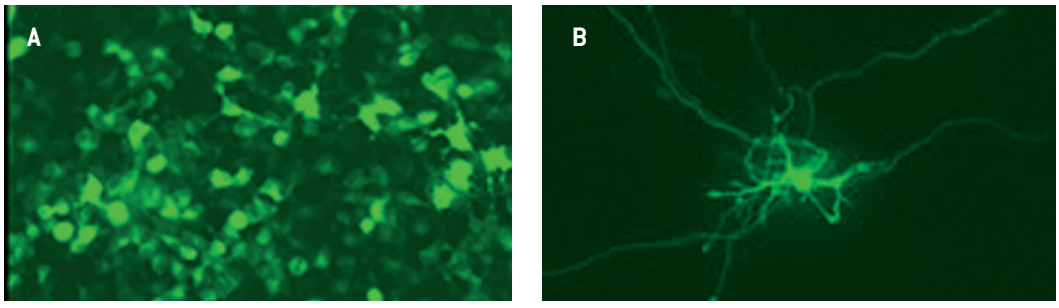
# Amaxa® Rat NSC Nucleofector® Kit

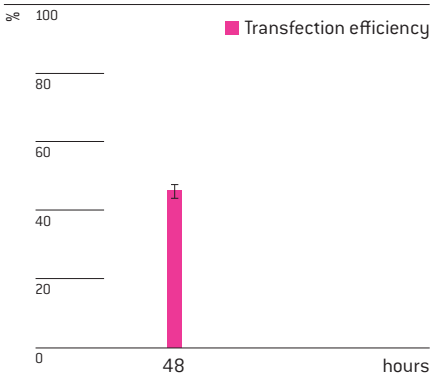
## For Rat Neural Stem Cells (NSC)

**Note** This protocol is optimized for primary NSC, isolated from cortex, midbrain or striatum of rat embryo (E14-16) and grown as neurospheres or adherently on fibronectin-coated plates. Cells grown under different conditions may require different settings for successful Nucleofection®.

### Example for Nucleofection® of primary rat NSC




**Primary NSC isolated from rat embryos (E14) were transfected with the Rat NSC Nucleofector® Kit, Program A-031 and a plasmid encoding enhanced green fluorescent protein eGFP under control of an EF1alpha promoter (pcDNAEF1-eGFP).** Cells were cultured with bFGF for 2 days post Nucleofection®, then for 5 additional days without bFGF to differentiate into neurons and astrocytes. Cells were analyzed 2 days (A) and 7 days(B) post Nucleofection® using fluorescence microscopy. Photograph courtesy of S.H. Lee, College of Medicine, Dept. of Biochemistry, Hanyang University, Seoul, South Korea.




**Average transfection efficiency of primary rat NSC.** Cells were transfected with program A-033 and 5 µg of a plasmid encoding enhanced green fluorescent protein eGFP under control of an EF1alpha promoter (pcDNAEF1-eGFP). Cells were analyzed 48 hours post Nucleofection® by fluorescence microscopy.

## Product Description

| | |
|---|---|
| Cat. No. | **VPG-1005** |
| Size (reactions) | 25 |
| Rat NSC Nucleofector® Solution | 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
- 24-well culture dish or culture system of your choice
- **Dissection solution**: 500 ml Hanks's BSS [Lonza; Cat. No. 10-508F], 5 ml penicillin/streptomycin, 3.5 ml 1 M HEPES (pH 7.3). We recommend sterilizing the solution by filtration and pre-cooling on ice before use
- **For coverslip preparation**: Glass coverslips [Marienfeld; 13 mm]; PBS; poly-L-ornithine solution (15 mg/ml, dissolved in PBS) [Sigma; Cat.No. P-3655]; fibronectin [Sigma; Cat.No. F-4759]
- **For detaching cells**: We recommend using trypsin/EDTA-HBS solution [Lonza; Cat.No. BE17-161E]
- **For trypsin inhibition**: 0.8 mg/ml trypsin inhibitor [Invitrogen; Cat.No. 17075-029] in dissection solution, sterilized by filtration
- **Culture medium I:** 500 ml DMEM/F12 (1 : 1) [Invitrogen; Cat.No. 11320] supplemented with N2 supplement (1 : 100) [Invitrogen; Cat. No. 17502-048], 780 µg D(+)-glucose, 36 mg L-glutamine, 0.85 g $NaHCO_3$, 12.5 mg Insulin [Sigma; Cat.No. 11882], 50 µg Transferrin [Sigma; Cat.No. T2036], 50 µl 1 M Putrescine [Sigma, Cat.No. P5780], 30 µl 0.5 mM Selenite [Sigma, Cat. No. S5261], 100 µl 0.1 mM Progesteron [Sigma; Cat.No. P8783]. Adjust to pH 7.2, add 5 ml penicillin/ streptomycin and sterilize the medium by filtration. Freshly add 10 – 20 ng/ml bFGF (basic fibroblast growth factor) [R&D; Cat.No. 233-FB]
- **Culture medium II (for differentiation)**: Culture medium I without bFGF, additionally 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®

### Preparation of coverslips for adherently grown cells

1.1 Put glass coverslips into a rack and boil in 100% ethanol (p.A.) for 5 minutes
1.2 Dry for 5 minutes under a laminar flow and autoclave
1.3 Place coverslips into a 24-well plate
1.4 Add 400 µl poly-L-ornithine solution and incubate in a humidified 37°C/5% $CO_2$ incubator overnight
1.5 Wash 2 x with PBS and dry
1.6 Prepare fibronectin solution (1 mg/ml) by carefully layering 2 ml sterile water over 2 mg fibronectin
1.7 Use after 30 minutes incubation at 4°C. Do not agitate

1.8 Incubate coverslips with fibronectin solution in a humidified 37°C/5% $CO_2$ incubator for at least 2 hours
1.9 Wash 2 x with sterile PBS

**Isolation & preparation of cells**

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

1.10 Separate heads from rat embryos (E14-16)
1.11 Dissect brains and extract cortices, midbrain and stria
1.12 Place tissue in culture medium I and mechanically dissociate cells using a fire-polished Pasteur pipette until the suspension is homogenous
1.13 Count cell numbers
1.14 Centrifuge for 5 minutes at 80xg
1.15 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
1.16 Seed cells on uncoated dishes if you like to grow them as neurospheres. Otherwise, seed cells on fibronectin-coated plates
1.17 Change medium every other day
1.18 Cells grown as neurospheres should be passaged 3 – 4 times before Nucleofection®
1.19 Cells grown on fibronectin-coated plates should be grown for 3 – 5 days until they reach 80 – 90% confluency. Carefully trypsinize cells before Nucleofection®. As soon as cells have detached, add trypsin inhibitor
1.20 Spin cells down for 5 minutes at 80xg
1.21 Carefully resuspend cells in culture medium I to get single cell suspension
1.22 Count cell numbers
1.23 Spin down the required number of cells (4 – 5 x $10^6$ cells per Nucleofection® sample) at 80xg for 5 minutes
1.24 Remove supernatant completely

## 2. Nucleofection®

**One Nucleofection® Sample contains**

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|---|
| 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 Rat NSC Nucleofector® Solution |

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare (coated coverslips in) 24-well plates by filling appropriate number of wells with 0.3 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% $CO_2$ incubator
2.3 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 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-031** (for best differentiation potential) or **A-033** (for high transfection efficiency and long term expression) (**A-31** 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 24-well plate (final volume 0.8 ml media per well) (altern.: 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
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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### References

1. Johe et al, [1996] Genes and Development 10: 3129-3140.

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