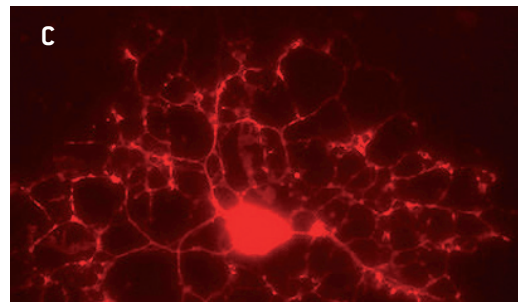
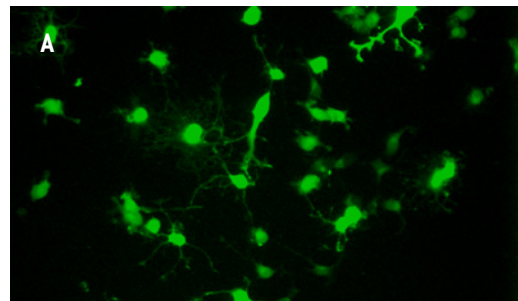


## Amaxa™ Nucleofector™ Protocol for Rat Oligodendrocytes

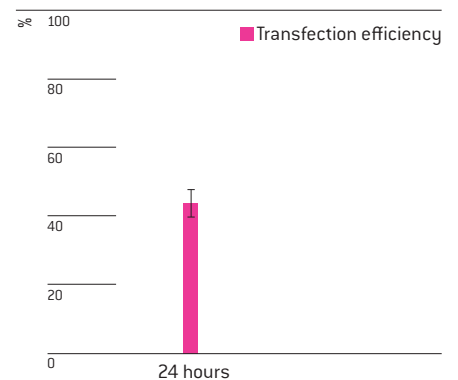
### For primary rat oligodendrocyte precursor cells

isolated from forebrains of newborn rats (p1) and cultivated for 17 days before Nucleofection™; flat bipolar cells; form multiple branches when mature

### Example for Nucleofection™ of rat oligodendrocyte precursor cells



Primary rat oligodendrocyte precursor cells were transfected using program 0-017 and 1 µg of a plasmid encoding the enhanced green fluorescent protein eGFP (A) or 0.25 µg of a plasmid encoding a histone 2B-tagged enhanced green fluorescent protein eGFP (B) which is predominantly located in the nucleus. Cells were analyzed 3 days post Nucleofection™ using fluorescence microscopy. In figure (C) cells were stained for the oligodendrocyte-specific marker MBP. Photograph courtesy of W. Deng, Children's Hospital, Boston, USA (A) and H. Colognato, Dept. of Pathology, Univ. of Cambridge, UK (B,C).



Average transfection efficiency of primary rat oligodendrocyte precursor cells 24 hours post Nucleofection™. Cells were transfected with program 0-017 and 1 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cell viability is around 60% 24 hours post Nucleofection™.

### Product Description

Recommended Kit: Basic Nucleofector™ Kit for Primary Mammalian Glial Cells

Cat. No.	VPI-1006
Size (reactions)	25
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
- 6-well culture dish or culture system of your choice
- **For detaching cells:** 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- **Culture medium:** DMEM containing 4.5 g/l glucose [Lonza; Cat. No. 12-733F, without L-glutamine and sodium pyruvate] supplemented with 4 mM L-glutamine [Lonza; Cat. No. 17-605E], 20% FCS, 1 mM sodium pyruvate [Lonza; Cat. No. 13-115E], 50 U/ml penicillin and 50 mg/ml streptomycin
- **Chemically defined medium (CDM):** DMEM supplemented with 25 mM D-glucose [Sigma; Cat. No. G-7021], 4 mM L-glutamine [Lonza; Cat. No. 17-605E], 1 mM sodium pyruvate [Lonza; Cat. No. 13-115E], 50 µg/ml human apo-transferrin [Sigma; Cat. No. T-5391], 5 µg/ml bovine pancreatic insulin [Sigma; Cat. No. I-1882], 30 nM sodium selenate [Sigma; Cat. No. S-8295], 10 nM hydrocortisone [Sigma; Cat. No. H-0135], 10 nM D-biotine [Sigma; Cat. No. B-4639], 1 mg/ml BSA [Sigma; Cat. No. A-4161]
- **Precursor cell maintaining medium:** Chemically defined medium supplemented with 10 ng/ml human PDGF-AA [Sigma; Cat. No. P-3076] and 10 ng/ml bFGF [Sigma; Cat. No. F-5392]
- Prewarm appropriate volume of CDM to 37°C (1.5 ml per sample)
- Appropriate number of cells (5 x 10<sup>6</sup> cells per sample; Minimal cell number: 1 x 10<sup>6</sup> cells [a lower cell number may lead to a major increase in cell mortality]. Maximum cell number 1 x 10<sup>7</sup>)

## 1. Pre Nucleofection™

**Note** This protocol only gives an outline for the isolation and culture of rat oligodendrocyte precursor cells. Please refer to more detailed protocols in the literature (see chapter 4) before starting the experiments.

### Isolation and cultivation of cells

- 1.1 Dissect forebrains of neonatal (P1) Sprague-Dawley rats, remove meninges
- 1.2 Dissociate brains by gentle trituration
- 1.3 Filter cell suspension through a 70 µm Nylon BD Falcon™ Cell Strainer [Becton Dickinson]
- 1.4 Grow cells in culture medium for 10 days in a humidified 37°C/5% CO<sub>2</sub> incubator. Change medium every 3 days
- 1.5 After confluency, deplete loosely attached microglia by shaking on a horizontal rotary platform for 1 hour at 200 rpm

- 1.6 Collect oligodendrocyte precursor cells by further vigorous shaking for 18 hours at 200 rpm
- 1.7 Plate cells on uncoated dishes for 30 minutes. Contaminating astrocytes and microglia will attach, oligodendrocyte precursor cells can be harvested by centrifugating the supernatant for 5 minutes at 80xg
- 1.8 Proliferate oligodendrocyte precursor cells for 7 days in precursor cell maintenance medium. Refresh half of medium every other day

### Trypsinization

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- 1.9 Add sufficient trypsin/EDTA solution to cover the cell layer and gently swirl the dish to ensure an even distribution of the solution
- 1.10 Briefly incubate at 37°C until the majority of cells has been dislodged. Avoid overexposure to trypsin/EDTA
- 1.11 Inactivate trypsin by adding culture medium

## 2. Nucleofection™

### One Nucleofection™ Sample contains

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5 x 10<sup>6</sup> cells

0.25 – 1 µg plasmid DNA (in 1 – 5 µl H<sub>2</sub>O or TE) or 1 µg pmaxGFP™ Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)

100 µl Nucleofector™ Solution

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- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
- 2.2 Prepare 6-well plates by filling appropriate number of wells with 1 ml of CDM and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.3 Remove the medium from the cell culture dishes
- 2.4 Harvest the cells by trypsinization (please see 1.9 – 1.11)
- 2.5 Count an aliquot of the cells and determine cell density
- 2.6 Centrifuge the required number of cells (5 x 10<sup>6</sup> cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
- 2.7 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 20 minutes), as this may reduce cell viability and gene transfer efficiency.

- 2.8 Combine 100 µl of cell suspension with 0.25 – 1 µg DNA, 1 µg pmaxGFP™ Vector 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 **0-017** or **A-033** (**0-17** or **A-33** for Nucleofector™ I Device). Please try both programs as transfection efficiency may vary depending on animal strain, preparation conditions etc.

- 2.11 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program by pressing the X-button
- 2.12 Take the cuvette out of the holder once the program is finished
- 2.13 Immediately add ~500 µl of the pre-equilibrated CDM to the cuvette and gently transfer the sample into the prepared 6-well plate (final volume 1.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

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### 3. Post Nucleofection™

- 3.1 Incubate the cells in humidified 37°C/5% CO<sub>2</sub> incubator. During the next days, precursor cells should differentiate into mature oligodendrocytes. The different stages may be characterized by immunochemical detection of stage-specific developmental markers: A2B5 (precursors), O4 (later stage-precursors), O1 (immature oligodendrocytes), and myelin basic protein (MBP, mature oligodendrocytes)
- 3.2 Depending on the gene, expression is often detectable after 8 – 16 hours already. It should persist for several days

## Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to:  
[www.lonza.com/nucleofection-citations](http://www.lonza.com/nucleofection-citations)

**For more technical assistance, contact our Scientific Support Team:**

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## References

1. P.N. Conn [Ed.], [1990] Methods in Neuroscience, Vol. 2, Academic Press
2. Deng et al., [2002] Brain Res. 929: 87-95

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Please note that the Amaxa™ Nucleofector™ Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

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