

## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit R

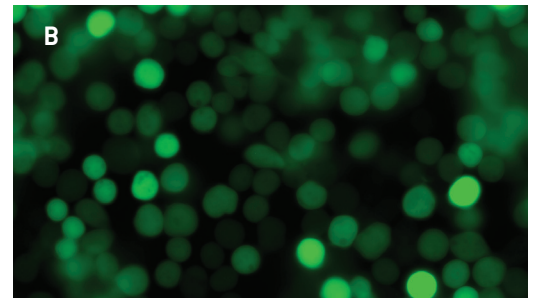
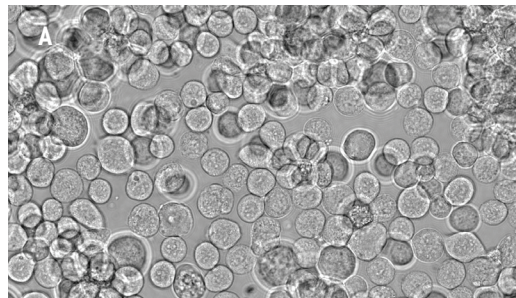
### For Sf9

Spodoptera frugiperda, pupal ovary; epithelial cells

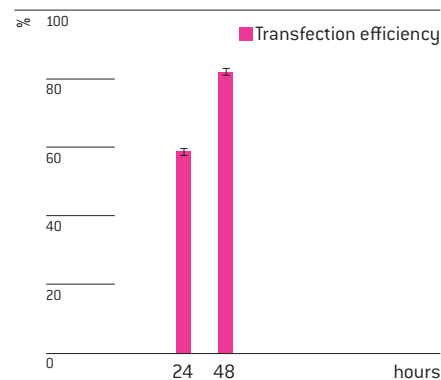
#### Important

The pmaxGFP<sup>®</sup> Vector provided in our Cell Line Nucleofector<sup>®</sup> Kit R is not expressed in insect cells! We strongly recommend an insect expression vector encoding a fluorescent protein or lacZ reporter as a positive control for your experiments [e.g. Novagen<sup>®</sup>'s pEx<sup>™</sup> Insect Cell Expression Plasmids].

#### Example for Nucleofection<sup>®</sup> of Sf9 cells



Sf9 cells were transfected with the Cell Line Nucleofector<sup>®</sup> Kit R, Program I-014 and 2 µg of an insect expression vector encoding maxGFP<sup>®</sup>. Cells were analyzed 48 hours post Nucleofection<sup>®</sup> using light (A) and fluorescence microscopy (B).



Average transfection efficiency of Sf9 cells. Sf9 cells were transfected with program I-014 and 2 µg of an insect expression vector encoding maxGFP<sup>®</sup>. Cells were analyzed 24 and 48 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell Viability [% PI negative] is around 80% 24 hours post Nucleofection<sup>®</sup>.

### Product Description

|  |  |
|--|--|
| Cat. No.   | VCA-1001   |
| Size (reactions)   | 25   |
| Cell Line Nucleofector <sup>®</sup> Solution R               | 2.25 ml (2.05 ml + 10% overfill)   |
| Supplement   | 0.5 ml (0.45 ml + 10% overfill)  |
| pmaxGFP <sup>®</sup> 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 <sup>®</sup> Solution, Supplement and pmaxGFP <sup>®</sup> Vector at 4°C. For long-term storage, pmaxGFP <sup>®</sup> Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector <sup>®</sup> Supplement is added to the Nucleofector <sup>®</sup> 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
- 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
- **Culture medium:** Grace's Insect Medium with L-glutamine, 500 mg/L calcium chloride, 2800 mg/L potassium chloride, 3330 mg/L lactalbumin hydrolysate, 3330 mg/L yeastolate, 90% [Gibco, Cat. No. 11595-030]; supplemented with 10% heat-inactivated fetal bovine serum (tested for insect cell culture: [SIGMA, Cat.No. F0643])
- Prewarm appropriate volume of culture medium to 28°C (2 ml per sample)
- Appropriate number of cells ( $1.5 \times 10^6$  or up to  $1 \times 10^7$  e.g. for protein production) per sample; lower or higher cell numbers may influence transfection results]

### 1. Pre Nucleofection®

#### Cell culture recommendations

- 1.1 Replace media every 3 – 4 days
- 1.2 Passage cells every 3 – 4 days with a subcultivation ratio of 1 : 3 to 1 : 6
- 1.3 Prepare subcultures by gently scraping
- 1.4 Seed out  $2 - 4 \times 10^6$  cells/T162 flask
- 1.5 Subculture 3 – 4 days before Nucleofection®
- 1.6 Use a culture flask with non-ventilated cap
- 1.7 Culture cells in a 28°C/100% air incubator without CO<sub>2</sub>
- 1.8 Centrifuge cells at 835xg for 6 minutes

### 2. Nucleofection®

#### One Nucleofection® Sample contains

1.5 x 10<sup>6</sup> cells (If transfection of high cell numbers is desired, e.g. for protein production, up to 1 x 10<sup>7</sup> cells can be transfected per Nucleofection® Reaction)

2 µg plasmid DNA (in 1 – 5 µl H<sub>2</sub>O or TE)

100 µl Cell Line Nucleofector® Solution R

- 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 1.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 28°C/100% air incubator
- 2.3 Count an aliquot of the cells and determine cell density
- 2.4 Centrifuge the required number of cells (1.5 x 10<sup>6</sup> or up to 1x 10<sup>7</sup> cells per sample) at 835xg for 6 minutes at room temperature. Remove supernatant completely
- 2.5 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.

- 2.6 Combine 100 µl of cell suspension with 2 µg DNA or other substrates
- 2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
- 2.8 Select the appropriate Nucleofector® Program I-014 (I-14 for Nucleofector® I Device)
- 2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
- 2.10 Take the cuvette out of the holder once the program is finished
- 2.11 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6-well plate (final volume 2 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 28°C/100% air incubator without CO<sub>2</sub> until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours

### 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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