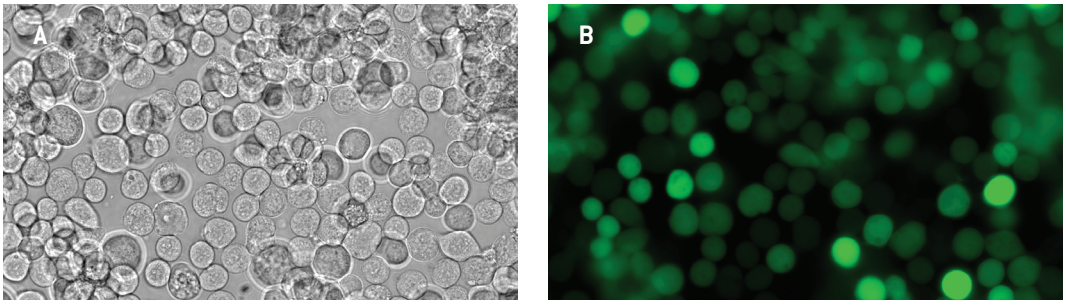
Amaxa® Cell Line Nucleofector® Kit R

For Sf9

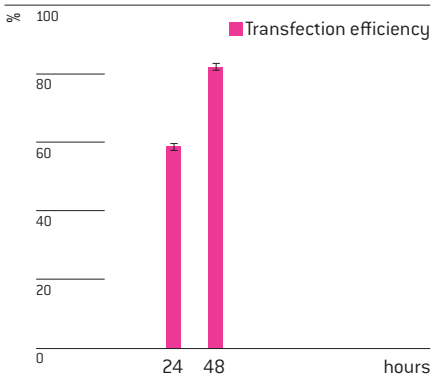
Spodoptera frugiperda, pupal ovary; epithelial cells

Important **The pmaxGFP® Vector provided in our Cell Line Nucleofector® 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®'s pIEx™ Insect Cell Expression Plasmids].**

Example for Nucleofection® of Sf9 cells



Sf9 cells were transfected with the Cell Line Nucleofector® Kit R, Program I-014 and 2 µg of an insect expression vector encoding maxGFP®. Cells were analyzed 48 hours post Nucleofection® 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®. Cells were analyzed 24 and 48 hours post Nucleofection® by flow cytometry. Cell Viability (% PI negative) is around 80% 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1001**
Size (reactions)	25
Cell Line Nucleofector® Solution R	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
- 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 x 10^6 or up to 1 x 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 x 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_2
1.8 Centrifuge cells at 835xg for 6 minutes

2. Nucleofection®

One Nucleofection® Sample contains

1.5 x 10^6 cells (If transfection of high cell numbers is desired, e.g. for protein production, up to 1 x 10^7 cells can be transfected per Nucleofection® Reaction)
2 µg plasmid DNA (in 1 – 5 µl H_2O 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 10^6 or up to 1x 10^7 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_2 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

For more technical assistance, contact our Scientific Support Team:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

Lonza Cologne AG
50829 Cologne, Germany

DCR-1017_2009-05