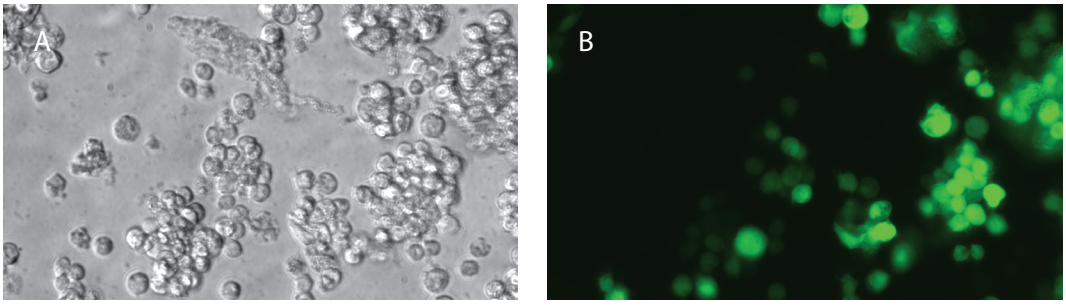
Amaxa® Cell Line Nucleofector® Kit V

For S2 (Schneider´s Drosophila Line 2 [D. Mel. (2); SL2])

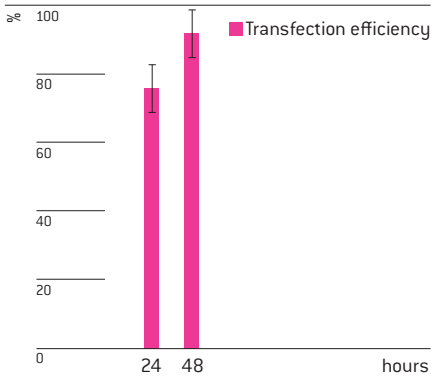
Drosophila melanogaster, embryo; epithelial cells

Note **The pmaxGFP® Vector provided in our Cell Line Nucleofector Kit V 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 or Invitrogen's DES® – Constitutive Kits].**

Example for Nucleofection® of S2 cells



S2 cells were transfected with the Cell Line Nucleofector® Kit V, Program G-030 and 2 µg of an insect expression vector encoding maxGFP®. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of S2 cells. S2 cells were transfected with program G-030 and 2 µg of an insect expression vector encoding maxGFP®. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell viability (% PI negative cells) is around 70% 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1003**
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**: Schneider's Drosophila Medium [Lonza Cat. No. 04-351Q]; fetal bovine serum (insect cells tested), 10% [Sigma; Cat. No. F0643]
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (2 x 10^6 cells per sample; up to 1 x 10^7, e.g. for protein production)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Passage cells every 2 – 3 days. Use a culture flask with non-ventilated cap! Culture cells in a 24°C/100% air incubator without CO_2!
1.2 Seed out 5 x 10^4 cells/cm^2
1.3 Subculture 3 – 4 days before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

2 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) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution V

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 1 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 24°C/100% air incubator without CO_2
2.3 Count an aliquot of the cells and determine cell density
2.4 Centrifuge the required number of cells (2 x 10^6 cells per sample) at 90xg for 10 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**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) 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 **G-030** (**G-30** 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 12-well plate (final volume 1.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 24°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:

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