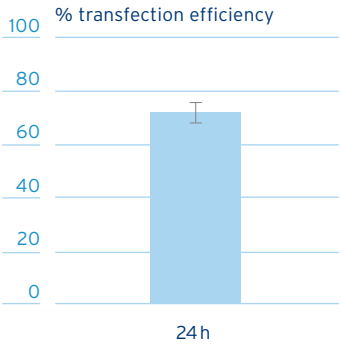
Cell Line Nucleofector® Kit L

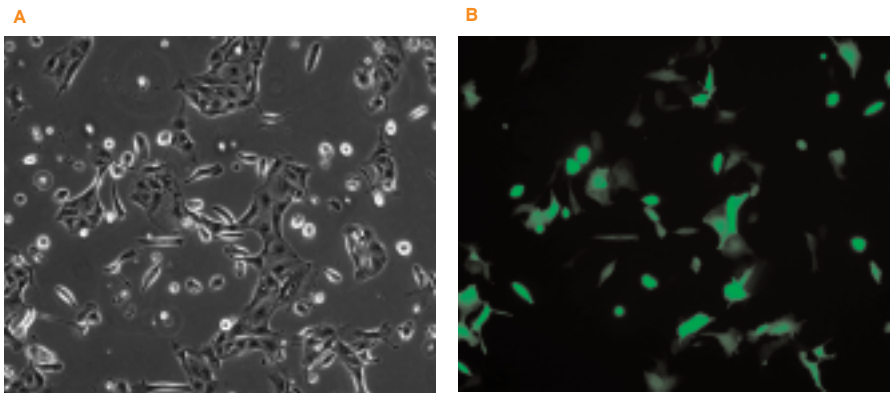
for MDCK Cell Line [ATCC]

Cell type		
	Origin	Dog kidney [ATCC; Cat. No. CCL-34; frozen vial].
	Morphology	Epithelial cell.



Average transfection efficiencies of MDCK cells. Cells were nucleofected with program **A-24 or A-024** and 5 µg of pmaxGFP. **24 hours** post nucleofection, the cells were analyzed by flow cytometry. Cell Viability (% PI negative) is around 83% after 24h post-nucleofection.

Example for nucleofection® of MDCK cells.



MDCK cells were nucleofected using the Cell Line Nucleofector Kit L, program **A-24 or A-024** and 5 µg of pmaxGFP. **24 hours** post nucleofection the cells were analyzed by fluorescence microscopy.

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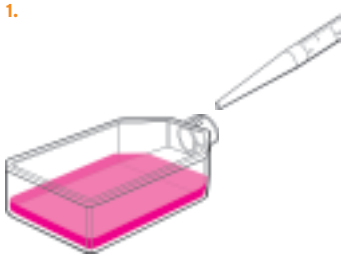
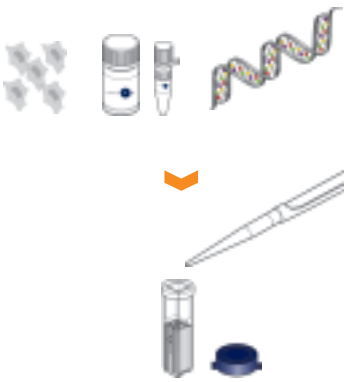
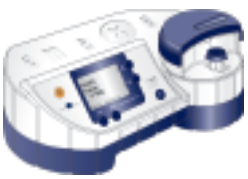
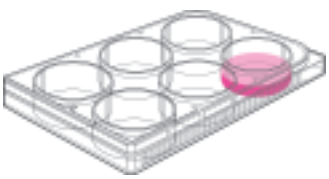
1 Procedure outline & important advice

	Procedure outline	Important advice
1. 	Culturing of cells before nucleofection. (For details see 3.3.)	› Replace medium every 2-3 days into a **new** culture vessel. › Passage 2-3 days before nucleofection. › Do not passage more than 20 times. › Only low centrifugation. › Resuspend cells very gently.
2. 	Combine the cells of interest, DNA or siRNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette. (For details see 3.5.)	**Contents of one nucleofection sample:** › 5 x 10^5 cells (optimal cell number) › 5 µg highly purified plasmid DNA (in max. 10 µl) or 0.5 - 3 µg siRNA › 100 µl Nucleofector Solution L Perform each sample separately to avoid storing the cells longer than 15 min in Nucleofector Solution L.
3. 	Choose the cell-type specific program. Insert the cuvette into the Nucleofector and press the "X" button to start the program. (For details see 3.5.)	› Optimal Nucleofector program: **A-24 or A-024**
4. 	Rinse the cuvette with culture medium using an amaxa certified pipette. Transfer the cells into the culture dish. (For details see 3.5.)	› Using an amaxa certified pipette, immediately remove sample from the cuvette with 500 µl prewarmed medium. › Transfer directly to 37°C.

2 Product description

Cat. No.	**VCA-1005**
Kit components	2.25 ml **Cell Line Nucleofector® Solution L**
	0.5 ml Supplement
	30 µg pmaxGFP™ (0.5 µg/µl in 10 mM Tris pH 8.0)
	25 certified cuvettes
	25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector Solution, Supplement and pmaxGFP at 4°C. For long term storage pmaxGFP is ideally stored at -20°C. The expiry date is printed on the Solution Box.

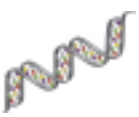
3 Protocol

3.1 › Required reagents

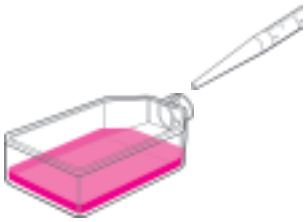
Medium Minimum essential medium (Eagle) with 2mM L-glutamine and Earle's BSS adjusted to contain 1.5g/L sodium bicarbonate, 0.1mM non-essential amino acids and 1.0mM sodium pyruvate, 90% [ATCC; Cat. No. 30-2003]; fetal bovine serum, 10% [ATCC; Cat. No. 30-2020].

Trypsin Treatment 2.5 mg/ml Trypsin; 1.0 mg/ml EDTA in PBS [Gibco; Cat. No. 15400-054: dilution 1:2] (10-15 min at 37°C).

3.2 › DNA preparation and quality



The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit]. The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN protocol.



3.3 › Cell culture

Culture conditions Replace medium every 2-3 days.

Passage interval Cells should be passaged every 2-3 days into a **new** culture vessel.

Seeding conditions A subcultivation ratio of 1:3 to 1:5 is recommended.

Culture conditions before nucleofection

- The cells should be preferably passaged 2-3 days before nucleofection.
- Use early passages for nucleofection.
- Do not passage more than 20 times.
- Only use low spin centrifugation.
- Resuspend cells very gently.

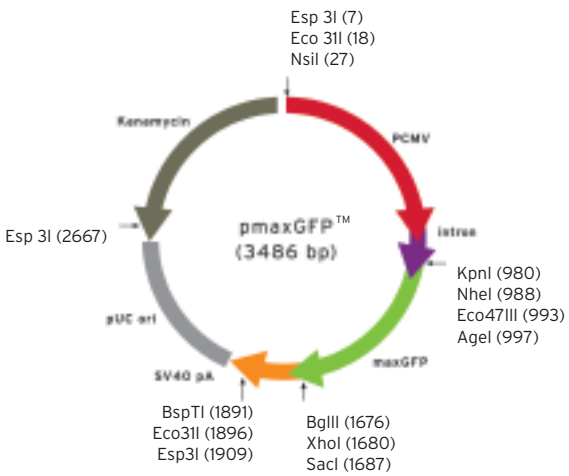
Note

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Normocin™ [Cat. No. VZA-1001], a new antibiotic formulation specifically developed to protect sensitive cell lines from mycoplasma infection and microbial contaminations. For more information and ordering info see **www.amaxa.com/antibiotics**.

3.4 › Important controls and vector information

Positive control

We strongly recommend establishing the Nucleofector technology with the positive control vector **pmaxGFP** as provided in this kit. pmaxGFP encodes the green fluorescent protein (GFP) from copepod *Pontellina p.* Just like eGFP expressing cells, maxGFP expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection or amount/purity of DNA on cell viabilty.

control 1 Recommended amount of cells in Nucleofector Solution with DNA but without application of the program (alternatively: untreated cells) **(Cells + Solution + DNA - program)**

control 2 Recommended amount of cells in Nucleofector Solution without DNA with application of the program **(Cells + Solution - DNA + program)**

Vector information

If using IRES sequences in your vectors, please remember that the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some cell types may not be expressed at all. As alternatives we suggest either: co-transfecting two (or more) plasmids, using one plasmid with each gene under the control of its own promoter, or making a GFP fusion.

3.5 › Nucleofection protocol

Preparation of Nucleofector Solution

Add 0.5 ml Supplement to 2.25 ml Nucleofector Solution and mix gently.
The Nucleofector Solution is now ready to use and is stable for 3 months at 4°C.
Note the date of addition on the vial.

One nucleofection sample contains

- **5 x 10^5 cells**
- **5 µg plasmid DNA (in 1 - 5 µl H_2O or TE) or 2 µg pmaxGFP or 0.5 - 3 µg siRNA**
- **100 µl Nucleofector Solution L**

For more details about the nucleofection of siRNA:
www.amaxa.com/RNAi

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **5 µg** DNA or **0.5 - 3 µg** siRNA for each sample.
3. Pre-warm the supplemented Nucleofector Solution **L** to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6-well plates by filling appropriate number of wells with 1 ml of culture medium containing supplements and serum. Pre-incubate plates in a humidified 37°C/5% CO_2 incubator.

5. Remove the medium from the cell culture. Wash cells once with PBS.
6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA (see Nucleofector Manual for details).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**5 x 10^5** cells per nucleofection sample) at 90xg at **room temperature** for 10 min. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature Nucleofector Solution **L** to a final concentration of **5 x 10^5 cells/100 µl**. Avoid storing the cell suspension longer than **15 min** in Nucleofector Solution as this reduces cell viability and gene transfer efficiency.

   Important: Steps 10-14 should be performed for each sample separately.

Nucleofection

10. Mix 100 µl of cell suspension with **5 µg** DNA or **0.5 - 3 µg** siRNA.
11. Transfer the nucleofection sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Select the appropriate Nucleofector program, **A-24 or A-024** (see Nucleofector Manual for details). Insert the cuvette into the cuvette holder (Nucleofector I : rotate carousel to final position) and press the "X" button to start the program.
13. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium and transfer the sample into the prepared 6-well plates. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.
14. Press the "X" button to reset the Nucleofector.
15. Repeat steps 10-14 for the remaining samples.

Cultivation after nucleofection

16. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6-well plates.
17. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following nucleofection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 4-8 hours. If this is not the case, the incubation period may be prolonged up to 24 hours.

4 Recommended literature

For an up-to-date list of all Nucleofector references, please refer to:
www.amaxa.com/citations

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