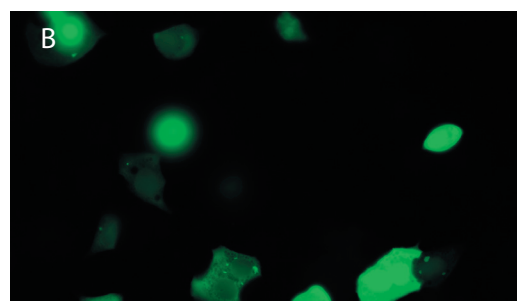
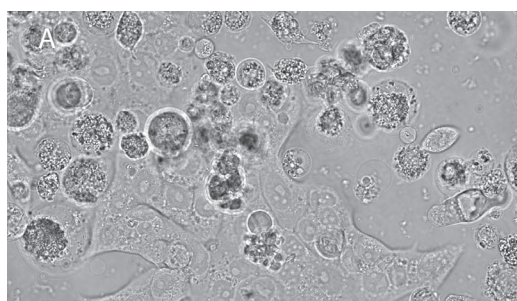


Amaxa[®] Cell Line Nucleofector[®] Kit V

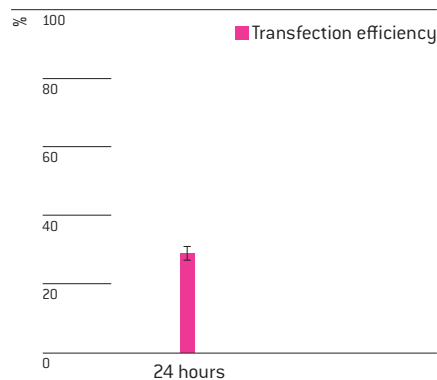
For Capan-1

Human pancreas adenocarcinoma; epithelial cells

Example for Nucleofection[®] of Capan-1 cells



Capan-1 cells were transfected with the Cell Line Nucleofector[®] Kit V, Program A-033 and 5 μg of pmaxGFP[®] Vector. Cells were analyzed 24 hours post Nucleofection[®] using light (A) and fluorescence microscopy (B).



Average transfection efficiency of Capan-1 cells. Capan-1 cells were transfected with program A-033 and 5 μg of pmaxGFP[®] Vector. Cells were analyzed 24 hours post Nucleofection[®] by flow cytometry. Cell viability [% PI negative cells] is around 78% 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 $\mu\text{g}/\mu\text{l}$ 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 collagen-coated culture dishes or culture system of your choice; collagen Type I (Sigma Cat. No. C-7661; for preparation of dish please see chapters 1.8-1.11
- **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:** Iscove's Modified Dulbecco's Medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate; fetal bovine serum, 20%
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10⁶ cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Cell culture recommendations

- 1.1 Replace media every 2 – 3 days
- 1.2 Passage cells every 3 – 4 days. A subcultivation ratio of 1 : 2 to 1 : 4 is recommended
- 1.3 Seed out 2 x 10⁶ cells/T162 flask
- 1.4 Subculture 4 days before Nucleofection® with a ratio of 1 : 2 – 1 : 4

Trypsinization

- 1.5 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
- 1.6 For harvesting, incubate the cells ~5 minutes at 37°C with indicated trypsinization reagent (please see required material)
- 1.7 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5% BSA once the majority of the cells (>90%) have been detached

Preparation of 12-well plates for cultivation

- 1.8 To prepare a 2.5 mg/ml collagen stock solution, dissolve the collagen in 0.2% sterile acetic acid by stirring at room temperature for 4 hours
- 1.9 Add 20 µl collagen stock and 1 ml 30% ethanol per well and coat 12-well plates for 24 hours at room temperature under a laminar flow
- 1.10 Wash 2 x with PBS
- 1.11 Remove PBS and fill with appropriate volume of culture medium

2. Nucleofection®

One Nucleofection® Sample contains

1 x 10 ⁶ cells
5 µg plasmid DNA (in 1 – 5 µl H ₂ O or TE) or 5 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 collagen coated plates by filling appropriate number of wells with 1 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
- 2.3 Harvest the cells by trypsinization (please see 1.5 – 1.7)
- 2.4 Count an aliquot of the cells and determine cell density
- 2.5 Centrifuge the required number of cells (1 x 10⁶ cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
- 2.6 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.

- 2.7 Combine 100 µl of cell suspension with 5 µg DNA, 5 µg pmaxGFP® Vector or 30 nM – 300 nM siRNA (3 – 30 pmol/sample) or other substrates
- 2.8 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
- 2.9 Select the appropriate Nucleofector® Program A-033 (A-33 for Nucleofector® I Device)
- 2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
- 2.11 Take the cuvette out of the holder once the program is finished
- 2.12 Incubate the sample in the cuvette for 10 minutes at room temperature
- 2.13 Add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 12-well collagen coated 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 humidified 37°C/5% CO₂ incubator 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

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