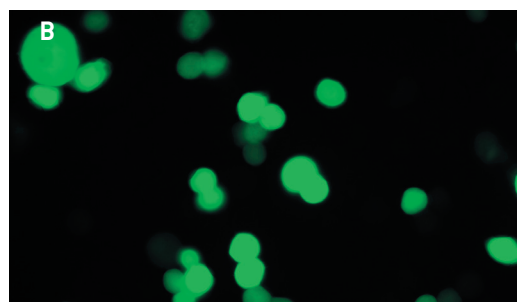


## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit C

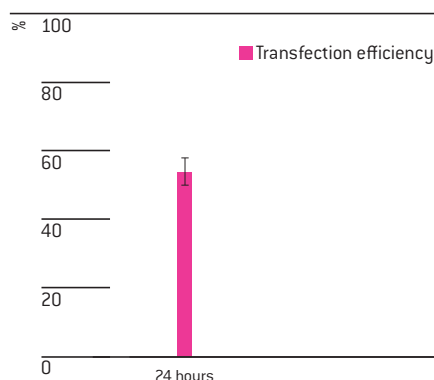
### For MDA-MB-453

Human breast mammary gland; epithelial cells

#### Example for Nucleofection<sup>®</sup> of MDA-MB-453 cells



MDA-MB-453 cells were transfected with the Cell Line Nucleofector<sup>®</sup> Kit C, Program X-001 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> using light (A) and fluorescence microscopy (B).



**Average transfection efficiency of MDA-MB-453 cells.**  
MDA-MB-453 cells were transfected with program X-001 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell Viability (compared to non-transfected control) is around 90% 24 hours post Nucleofection<sup>®</sup>.

### Product Description

Cat. No.	VCA-1004
Size (reactions)	25
Cell Line Nucleofector <sup>®</sup> Solution C	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 [Software requirements: version V2.3 or higher for Nucleofector® I Device; version S3-4 or higher for Nucleofector® II 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
- 6-well culture dish or culture system of your choice
- **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:** Leibovitz's L-15 medium with 2 mM L-glutamine, 90%; fetal bovine serum, 10% Culture without CO<sub>2</sub>
- Prewarm appropriate volume of culture medium to 37°C [1.5 ml per sample]
- Appropriate number of cells [1 x 10<sup>6</sup> 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. Culture without CO<sub>2</sub>
- 1.2 Passage cells 2 – 3 times a week
- 1.3 Maintain cultures with a subcultivation ratio of 1 : 2 – 1 : 3
- 1.4 Seed out 1.5 – 2.5 x 10<sup>4</sup> cells/cm<sup>2</sup>
- 1.5 Subculture 2 – 3 days before Nucleofection® with a ratio of 1 : 2
- 1.6 Please use low spin centrifugation (90xg)

### Trypsinization

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

### 2. Nucleofection®

#### One Nucleofection® Sample contains

1 x 10 <sup>6</sup> cells
2 µg plasmid DNA (in 1 – 5 µl H <sub>2</sub> O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution C

- 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 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/100% air incubator without CO<sub>2</sub>
- 2.3 Harvest the cells by trypsinization (please see 1.7 – 1.9)
- 2.4 Count an aliquot of the cells and determine cell density
- 2.5 Centrifuge the required number of cells (1 x 10<sup>6</sup> 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 2 µg DNA, 2 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 X-001 (X-01 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 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 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 37°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)

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