Amaxa® Cell Line Nucleofector® Kit V

For PC-3

Human prostate adenocarcinoma; epithelial cells

Example for Nucleofection® of PC-3 cells

PC-3 cells were transfected with the Cell Line Nucleofector® Kit V, Program T-013 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).

Average transfection efficiency of PC-3 cells. PC-3 cells were transfected with program T-013 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 and 48 hours post Nucleofection® by flow cytometry. Cell viability is around 60% 48 hours post Nucleofection®.

Product Description

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>VCA-1003</th>
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<tbody>
<tr>
<td>Size (reactions)</td>
<td>25</td>
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<tr>
<td>Cell Line Nucleofector® Solution V</td>
<td>2.25 ml [2.05 ml + 10% overfill]</td>
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<tr>
<td>Supplement</td>
<td>0.5 ml [0.45 ml + 10% overfill]</td>
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<tr>
<td>pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)</td>
<td>30 µg</td>
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<tr>
<td>Certified cuvettes</td>
<td>25</td>
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<tr>
<td>Plastic pipettes</td>
<td>25</td>
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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
- 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: formulated F-12K medium (Kaighn’s Modification of Ham’s F-12 Medium), supplemented with 10% FCS
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10^6 cells per sample; minimum recommended cell number: 5 x 10^5 cells per sample; a lower cell number leads to a major cell loss during Nucleofection®; maximum cell number: 2 x 10^6 cells per sample)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media every 2 – 3 days
1.2 Passage cells every 2 – 3 days
1.3 Seed out 3 x 10^4 cells/cm²
1.4 Subculture 2 – 3 days before Nucleofection®
1.5 Optimal confluency for Nucleofection®: 70 – 90%

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.7 For harvesting, incubate the cells ~15 – 20 minutes at 37°C with indicated trypsinization reagent (please see required material)
1.8 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 10⁶ cells
- 0.5 – 5 µg plasmid DNA (in 1 – 5 µl H₂O 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 6-well plates by filling appropriate number of wells with 1 ml of supplemented culture media and pre-incubate/equilbrate plates in a humidified 37°C/5% CO₂ incubator
2.3 Optional: Harvest the cells by trypsinization (please see 1.6 – 1.8)
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 100xg 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 0.5 – 5 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 T-013 (T-13 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 humidified 37°C/5% CO₂ incubator until analysis. Cells need at least 5 hours to reattach. Therefore, we recommend analyzing expression after 24 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:

<table>
<thead>
<tr>
<th>USA/Canada</th>
<th>Europe and Rest of World</th>
</tr>
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<tbody>
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<td>E-mail: <a href="mailto:scientific.support.eu@lonza.com">scientific.support.eu@lonza.com</a></td>
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50829 Cologne, Germany

Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

The Nucleofector® Technology, comprising Nucleofection® Process, Nucleofector® Device, Nucleofector® Solutions, Nucleofector® 96-well Shuttle® System and 96-well Nucleocuvette® plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne AG.

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