Amaxa® Cell Line Nucleofector® Kit R

For LNCaP

Human prostate carcinoma; epithelial cells

Example for Nucleofection® of LNCaP cells

LNCaP cells were transfected with the Cell Line Nucleofector® Kit R, Program T-009 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).

Average transfection efficiency of LNCaP cells. LNCaP cells were transfected with program T-009 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 and 48 hours post Nucleofection® by flow cytometry. Cell Viability (% PI negative) is around 70% 48 hours post Nucleofection®.

Product Description

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>VCA-1001</th>
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<tbody>
<tr>
<td>Size (reactions)</td>
<td>25</td>
</tr>
<tr>
<td>Cell Line Nucleofector® Solution R</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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</table>

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
- PolyLysine-coated 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
- Culture medium: RPMI 1640 supplemented with 10% CBS Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (2 x 10⁶ cells per sample; minimum cell number: 1 x 10⁶. Maximum cell number 5 x 10⁶. Lower cells numbers lead to reduced transfection efficiencies)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media every 3 – 4 days
1.2 Passage cells every 3 – 4 days. Cells should not be passaged more than 15 times
1.3 Seed out 3 x 10⁴ cells/cm²
1.4 Subculture 2 – 4 days before Nucleofection®. Cells should be nucleofected after reaching around 60 – 80% confluency

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 ~10 – 15 minutes at 37°C with indicated trypsinization reagent (please see required material)
1.7 Neutralize trypsinization reaction with supplemented culture medium once the majority of the cells (>90%) have been detached
2. Nucleofection®

One Nucleofection® Sample contains

- 2 x 10⁶ cells
- 2 – 5 µg plasmid DNA (in 1 – 5 µl H₂O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA
  (3 – 30 pmol/sample)
- 100 µl Cell Line Nucleofector® Solution R

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare polyLysine-coated 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/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 (2 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 2 – 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-009 (T-09 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 polyLysine-coated 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. Gene expression or down
  regulation, respectively, is often detectable after only 4 – 8 hours
Optimized Protocol for LNCaP Cells

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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