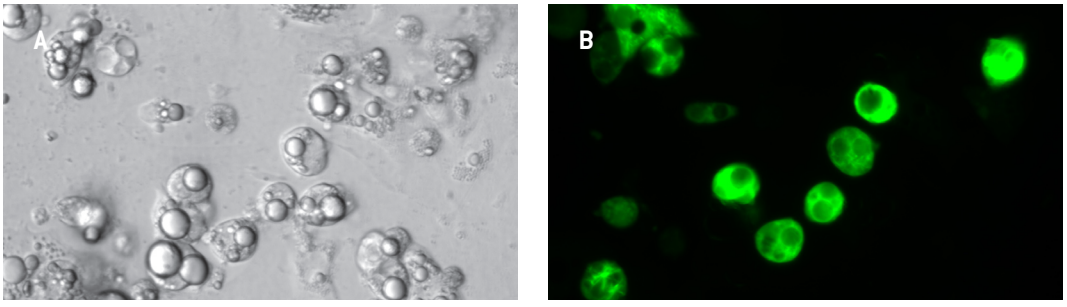
Amaxa® Cell Line Nucleofector® Kit L

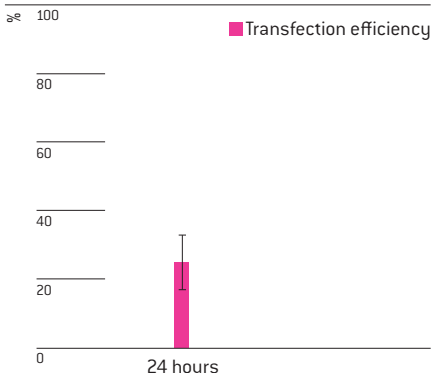
For 3T3-L1 (adipocytes) [ATCC® CL-173™, cryopreserved]

Mouse embryonal fibroblast, differentiated into adipocytes; Fibroblast-like cells before differentiation; adipocyte-like cells after differentiation;

Example for Nucleofection® of 3T3-L1 (adipocytes)cells



3T3-L1 (adipocytes)cells (ATCC® CL-173™) were transfected with the Nucleofector® Kit L, Program A-033 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of 3T3-L1 (adipocytes) cells. 3T3-L1 (adipocytes) cells (ATCC® CL-173™) were transfected with program A-033 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell Viability (compared to non-transfected control) is around 90% 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1005**
Size (reactions)	25
Cell Line Nucleofector® Solution L	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® 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® 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 coating of plates**: Prepare a 2.5 mg/ml collagen stock solution by dissolving collagen (Type I; Sigma Cat. No. C-7661) in 0.2% sterile acetic acid. Stir at room temperature for 4 hours. Alternatively, prepare a 2% gelatin solution (20 mg/ml, gelatin solution type B from bovine skin [Sigma, Cat. No. G1393])
- **For detaching cells**: 2.5 mg/ml Trypsin and 1.0 mg/ml EDTA in PBS (5x) and supplemented culture media or PBS/0.5% BSA
- **Culture medium**: DMEM with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose [ATCC®; Cat. No. 30-2002], 90%; calf bovine serum, 10% [ATCC®; Cat. No. 30-2030]
- **Differentiation medium I**: DMEM [ATCC®; Cat. No. 30-2002], 10% fetal bovine serum [ATCC®; Cat. No. 30-2020], 0.86 µM (5 µg/ml) human insulin [Sigma-Aldrich; Cat.No. I 9278]; 0.25 µM dexamethasone [Sigma-Aldrich; Cat.No. D-1756] and 0.5 mM Iso-butylmethyl-xanthine (IBMX) [Sigma-Aldrich; Cat.No. I 5879]
- **Differentiation medium II**: DMEM [ATCC®; Cat. No. 30-2002], 10% fetal bovine serum [ATCC®; Cat. No. 30-2020], 0.86 µM (5 µg/ml) human insulin
- **Differentiation medium III**: Differentiation medium II without human insulin. This medium is used post Nucleofection®
- **Wash medium**: Differentiation medium III supplemented with 4% glycerol
- Prewarm appropriate volume of differentiation medium III to 37°C (2.5 ml per sample)
- Appropriate number of cells (2 x 10^6 cells per sample; a lower or higher cell number may lead to a major increase in cell mortality)

1. Pre Nucleofection®

Cell culture recommendations for undifferentiated 3T3-L1 cells

1.1 Subculture cells 3 times a week. Renew medium every 2 – 3 days
1.2 Passage cells at 80 % confluency. Avoid 100% confluency!
1.3 Seed out 4 x 10^5 cells/75 cm^2 flask (see ATCC® protocol)

Differentiation

1.4 Cells should be seeded with a density of 5 x 10^5 cells per 162 cm^2 flask
1.5 Let cells grow to 80 – 100% confluency within 5 days and start differentiation at this point
1.6 To start differentiation change culture medium to differentiation medium I (day 0 of differentiation)

1.7 On day 2 of differentiation renew differentiation medium I
1.8 On day 4 of differentiation switch to differentiation medium II for another 2 days
1.9 On day 6 of differentiation switch to differentiation medium III for another 4 days
1.10 Cells should be differentiated for 10 days. For Nucleofection® only use 10 day differentiated 3T3-L1 adipocytes

Note **Cells differentiated for less than 10 days can also be transfected using the same Nucleofection® Parameters as described in this protocol. However, differentiation of less than 10 days will result in incompletely differentiated adipocytes.**

Trypsinization

1.11 Remove media from the cultured cells

Note **To save floating cells from the supernatant, store the supernatant in 50 ml tubes, add 4% glycerol and mix vigorously. This stored supernatant can be used to neutralize trypsinization.**

1.12 Wash cells once with PBS; use at least same volume of PBS as culture media
1.13 For harvesting, incubate the cells ~10 minutes at 37°C with indicated trypsinization reagent (please see required material)
1.14 Neutralize trypsinization reaction with wash medium
1.15 Centrifuge the cells at 90xg for 10 minutes at room temperature. Remove supernatant completely
1.16 Wash cells with 10 ml wash medium

Preparation of collagen-coated 6-well plates for cultivation after Nucleofection®

1.17 Add 20 µl collagen stock and 1 ml 30% ethanol per well and coat 6-well plates for 24 hours at room temperature under a laminar flow
1.18 Wash 2 x with PBS
1.19 Dry plates for about 15 minutes under a laminar flow

Alternative: Gelatin-coated plates

1.20 Prewarm a 2% gelatin solution at 37°C for 30 minutes
1.21 Then dissolve gelatin in PBS at 1 : 8 (final conc.: 2.5 mg/ml)
1.22 Add 2 ml of diluted gelatin to each well and coat 6-well plates for 30 minutes at room temperature under laminar flow
1.23 Exhaust non-coated gelatin and dry plates for about 15 minutes under a laminar flow

2. Nucleofection®

One Nucleofection® Sample contains
2×10^6 cells
2 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution L

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Use 10 day differentiated 3T3-L1 adipocytes for Nucleofection®
2.3 Prepare collagen-coated 6-well plates by filling appropriate number of wells with 2 ml of Differentiation Medium III [DMEM, FCS] and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator for at least 30 minutes
2.4 Harvest the cells by trypsinization (please see 1.11 – 1.16)
2.5 Centrifuge the cells at 90xg at room temperature for 10 minutes. Discard supernatant
2.6 Count an aliquot of the cells and determine cell density
2.7 Centrifuge the required number of cells (2×10^6 cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.8 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.**

2.9 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
2.10 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.11 Select the appropriate Nucleofector® Program **A-033** (**A-33** for Nucleofector® I Device)
2.12 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.13 Take the cuvette out of the holder once the program is finished
2.14 Immediately add ~500 µl of the pre-equilibrated differentiation medium III to the cuvette and gently transfer the sample into the prepared 6-well plate (final volume 2.5 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% CO_2 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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