BioResearch

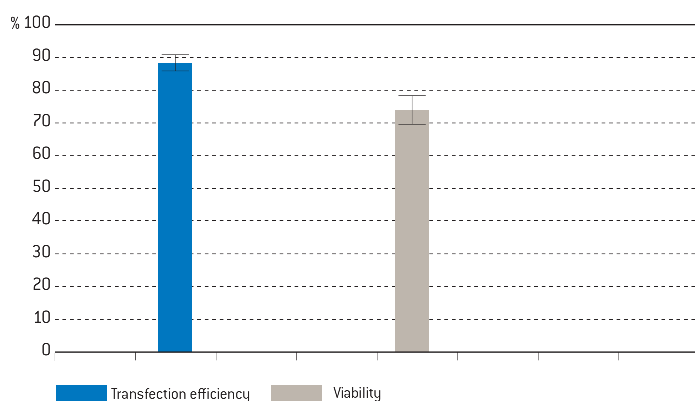
Lonza

Amaxa™ 96-well Shuttle™ Protocol for Mouse Embryonic Fibroblasts (MEF)

Cell Description

Validated to work with primary (non-immortalized) mouse embryonic fibroblasts from Lonza (Cat. No. M-FB-481). Fibroblastoid adherend cells.

Example for 96-well Nucleofection™ of MEF Cells



Transfection efficiency of MEF cells 24 hours post Nucleofection™. 1.0 x 10^5 cells were transfected with program 96-CZ-167 using 0.4 µg pmaxGFP™ Vector. Cells were analyzed 24 hours post Nucleofection™ using a FACSCalibur™ [Becton Dickinson]. Cell viability was measured with the ViaLight™ Plus Bioassay Kit; Lonza, Cat. No. LT07–221).

Product Description

Cat. No.	V4SP–4096
Size (reactions)	1×96
P4 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SP–4960
Size (reactions)	10×96
P4 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	10

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.

Note

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Required Material

Note

Please make sure that the entire supplement is added to the Nucleofector™ Solution.

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ plate(s)
- Nucleocuvette™ compatible tips: epT.I.P.S. [US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266], Matrix TallTips™ [Matrix Technologies Corp., Cat. No. 7281] or LTS Tips [Rainin Instrument, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S]. Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ wells without getting stuck
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note

Volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- 96-well culture plates or culture plates of your choice
- **Culture medium:** DMEM with 4.5 g/l glucose and 4 mM glutamine [Lonza; Cat. No. 12–604F] supplemented with 10% fetal bovine serum (FBS) (Lonza; Cat. No. 14-503E), 100µg/ml streptomycin and 100U/ml penicillin
- For detaching cells: HEPES (CC-5022), Trypsin–Versene Mixture (17–161E) and Trypsin Neutralizing Solution (TNS) [Lonza; Cat. No. CC–5002]
- Prewarm appropriate volume of culture medium to 37°C (140 µl per sample)
- Appropriate number of cells (1 x 10^5 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note

Transfection results may be donor-dependent.

Cell Culture Recommendations

1.1 Seeding conditions: 8–10 x 10^3 cells/cm^2
1.2 Cells should be passaged after reaching 70–80% confluency; replace media every two days
1.3 Optimal confluency before Nucleofection™ is 60–70%
1.4 Do not use cells after passage number 4 as this may result in substantially lower gene transfer efficiency and viability

Trypsinization

1.5 Remove media from the cultured cells and wash cells once with Hepes; use at least same volume of Hepes as culture media
1.6 Cells are very sensitive to trypsin treatment. For harvesting, incubate the cells 1–3 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.7 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached. Do not incubate the cells in TNS longer than 10 minutes

2. Nucleofection™

One Nucleofection™ Sample Contains

- 1 x 10^5 cells
- 0.4–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300nM siRNA (0.6–6 pmol/sample)
- 20 µl P4 Primary Cell 96-well Nucleofector™ Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see manual "Nucleofector™ 96-well Shuttle System)
2.3 Select the appropriate 96-well Nucleofector™ Program **96–CZ–167**
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 60 µl* (see note at the end of this chapter) for one well of a 96-well plate and pre–incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.5 Pre–warm an aliquot of culture medium to 37°C (80 µl per sample*)
2.6 Prepare 0.4–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or

30 nM–300 nM siRNA (0.6–6 pmol/sample)

2.7 Harvest the cells by trypsinization (please see 1.5–1.7)

2.8 Count an aliquot of the cells and determine cell density

2.9 Centrifuge the required number of cells (1 x 10^5 cells per sample) at 200xg for 10 minutes at room temperature. Remove supernatant completely

2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A. One or several substrates (DNAs or RNAs) in multiples:

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B. Multiple substrates (e.g. library transfection):

- Pipette 20 µl of cell suspension into each well of a sterile U– or V–bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre–dispense each cell suspension into a sterile round–bottom 96-well plate or to pipet from a pipetting reservoir for multi–channel pipettes. Use a multi–channel or single–channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

2.11 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.12 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.13 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.14 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer.

2.15 Incubate Nucleocuvette™ Plate for 10 min at room temperature

2.16 Resuspend cells with 80 µl* (recommendation for 96-well plates) or desired volume of pre–warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times.

2.17 Plate desired amount of cells in culture system of your choice.

Recommendation for 96-well plates: Transfer 40 µl of resuspended cells to 60 µl pre–warmed medium prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases. However, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

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

D4SP-4003_2011-03

Additional Information

Up–To–Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

Technical Assistance and Scientific Support

USA/Canada
Tel 800 521 0390 (toll-free)
Fax 301 845 8338
scientific.support@lonza.com

Europe and Rest of World
Tel +49 221 99199 400
Fax +49 221 99199 499
scientific.support.eu@lonza.com

www.lonza.com

Lonza Cologne GmbH–50829 Cologne, Germany

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