BioResearch

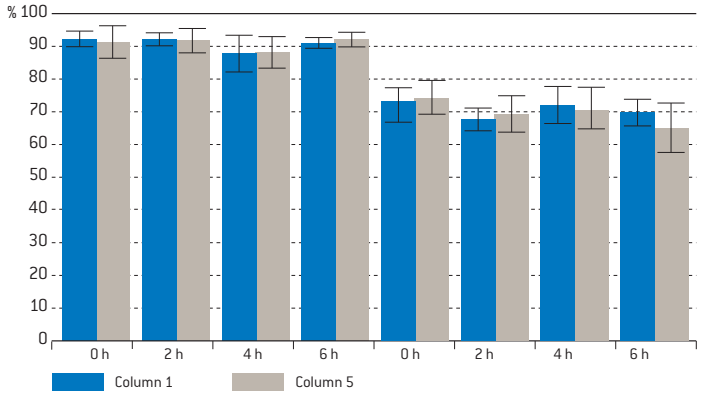
Lonza

Amaxa™ 96-well Shuttle™ Automation Protocol for Jurkat (ATCC®)

Cell Description

Human T cell leukemia. We strongly recommend using Jurkat clones E6.1 [ATCC® TIB-152™; frozen vial]. Round single cells.

Example for 96-well Nucleofection™ of Jurkat cells



Efficiency and Viability depending on the incubation time pre-Nucleofection™ on different positions of the Nucleocuvette™ plate. Jurkat cells were nucleofected with program 96-CM-137 and 1 µg of pmaxGFP™. 48 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option. Cell viability was determined using PI-staining.

Product Description

Recommended Kit(s)—Jurkat Automation 96-well Nucleofector™ Kit

Cat. No.	VHCA-4001
Size (reactions)	customized
Kit components per 1 x 96 unit:	
*Volumes will be customized depending on the required overfill to cover void volumes etc.	
Cell Line 96-well Nucleofector™ Solution AA1	1.035 ml*
Cell Line 96-well Nucleofector™ Solution AA2	1.265 ml*
Supplement	0.23 ml*
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ plate(s)	1

* Volumes will be customized depending on the required overfill to cover void volumes etc.

Storage and Stability

Store Solution, Supplement at 4°C, pmaxGFP™ at 4°C for short term storage or at -20°C for long term storage. The expiry date is printed on the Solution Box. The mixture of Nucleofector™ Solutions AA1, AA2 and Supplement should be prepared only directly prior to the experiment.

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 mix the entire content of Nucleofector™ Solutions AA1 and AA2 with supplement directly before the experiment. The 96-well Nucleofector™ Solution AA is then ready for use. If the solutions are supposed to be used partially i.e. to split up the experiment, mix Nucleofector™ Solution AA1, AA2 and supplement in a ratio of 1.035 / 1.265 / 0.23 ml.

- 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.

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 96-well culture plates or culture plates of your choice
- Culture medium: RPMI 1640 medium with 2 mM L-glutamine adjusted
- to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES and 1.0 mM sodium pyruvate, 90 % (ATCC®, Cat. No. 30-2001); fetal bovine serum, 10 % (ATCC®, Cat. No. 30-2020)
- Prewarm appropriate volume of culture medium to 37°C (230 µl per sample)
- Appropriate number of cells: 6 x 10 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 Replace media 3–4 times a week
1.2 Cells should be passaged at a density of 5 x 10^5 cells/ml. We recommend seeding 1 x 10^5 cells/ml.
1.3 Subculture 2 days before Nucleofection™; cells should be grown to a density of 3 x 10^5 cells/ml before Nucleofection™.

2. Nucleofection™

One Nucleofection™ Sample Contains

- 6 x 10^4 cells
- 0.2 µg–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 1 µg pmaxGFP™ Vector or 30–300nM siRNA (0.6–6 pmol/sample)
- 20 µl Nucleofector™ Solution AA (see required material)

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 device and software manuals)
2.3 Select the appropriate 96-well Nucleofector™ Program 96-CM-137
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 100 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 (100 µl per sample*)
2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30 nM–300 nM siRNA (0.6–6 pmol/sample)
2.7 Count an aliquot of the cells and determine cell density
2.8 Centrifuge the required number of cells (6 x 10^4 cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.9 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.

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

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

2.12 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)

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

2.14 Resuspend cells with 100 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.

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 100 µl of resuspended cells to 100 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 cells in a humidified 37°C/5% CO_2 incubator. Following Nucleofection™, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 4–8 hours. If this is not the case, the incubation period may be prolonged to 24 or 48 hours.

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Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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