BioResearch

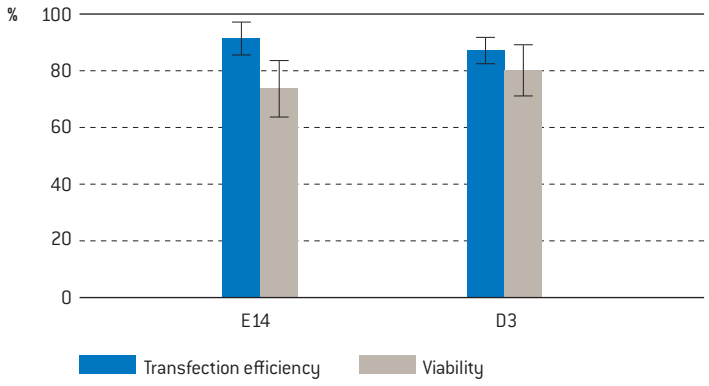
Lonza

Amaxa™ HT Nucleofector™ protocol for mouse ES cells

Cell description

This protocol is designed for mouse embryonic stem (ES) cells. Cells derived from mouse blastocysts; round cells, growing in clumps.

Example for Nucleofection™ of mouse embryonic stem cells



Average transfection efficiency of mouse ES cells 24 hours post Nucleofection™. 5×10^4 mouse ES cells were transfected with program CG-104-AA and 0.4 µg of pmaxGFP™ vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option. Cell viability is given in percent compared to non-transfected control.

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2×384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-3010
Size (reactions)	10×384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well 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.

Notes

- This protocol is meant to provide an outline for the handling and the Nucleofection™ of mouse ES cells. It has been optimized for mouse ES cell lines ES-E14TG2a (ATCC®-CRL-1821™) and ES-D3 (ATCC®-1934™) but will work with other cells lines as well.
- Experimental results and cell viability may vary depending on the mouse ES cell line used.
- HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, 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.

- HT Nucleofector™ System
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well Nucleocuvette™ plate(s)
- 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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
- 96-well culture plates or culture plates 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
- Mouse ES culture medium: DMEM (ATCC; Cat. No. 30-2002) supplemented with 10 % FCS (ES Cell Qualified) (Invitrogen; Cat. No. 16141-079), 1000 U/ml ESGRO LIF (Chemicon; Cat. No. ESG1107), 0.1 mM 2-Mercaptoethanol and 1% Pen/Strep (100 µg/ml streptomycin, 100 U/ml penicillin)
- MEF feeder cells: We recommend using irradiated mouse embryonic fibroblasts (MEF) (STO IRR, ATCC® 56-X™) or STO MEF feeder cells (ATCC® CRL-1503™) inactivated by mitomycin-C treatment (see chapter1)
- For MEF inactivation: Mitomycin-C (Sigma); Ca^{2+}- and Mg^{2+}-free HBSS
- MEF Culture Medium: DMEM (ATCC 30-2002) + 10 % FCS (ES Cell Qualified) (Invitrogen 16141-079) + 1 % P/S (100 µg/ml streptomycin, 100 U/ml penicillin)
- Pre-warm appropriate volume of mouse ES culture medium to 37°C (192 µl per sample)
- Appropriate number of cells (5×10^4 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Cell Culture

Note
Mouse ES cells are cultured on irradiated or inactivated mouse embryonic fibroblast (MEF) feeder cells in gelatine-coated plates.

Culture conditions for MEFs before inactivation

1.1 Replace medium 2 to 3 times per week cells should be passaged 3 times per week with a subcultivation ratio of 1 : 3 to 1 : 10

Inactivation of MEF feeder cells

1.2 Prepare a mitomycin-C stock solution 1 mg/ml stock, filter sterilized it and store sterile solution at 4°C protected from light
1.3 Expand a vial of MEFs as described by ATCC®
1.4 Add 1 mg/ml mitomycin-C stock solution to the medium to a final concentration of 10 µg/ml. Return plates to the incubator for 2 to 3 hours
1.5 Rinse plates twice with 10 to 15 ml Ca^{2+}- and Mg^{2+}-free HBSS Trypsinize as if passaging
1.6 Add an equal volume of MEF culture medium without penicillin/streptomycin
1.7 Count and freeze cells

Preparation of Gelatin Coated Plates

1.8 Add 0.5 grams gelatin to 500 ml endotoxin-free water (0.1 % solution). Gelatin will not be soluble. Do not use glass bottles that have seen detergent. Glass bottles should be cleaned with NaOH when first obtained, and then dedicated to sterile gelatin solution. Do not allow water or gelatin solution to sit unsterilized for any longer than a couple of hours before autoclaving
1.9 Autoclave for 30 minutes. Gelatin will solubilize and remain a liquid. Store at room temperature
1.10 At least one hour prior to plating of irradiated MEF, coat plate/flask with gelatin solution by placing at least 100 µL per well of gelatin into a 96-well plate (or 3 ml gelatin solution into a 25 cm^2 flask). Tilt plates/flask in several directions so that liquid covers the entire surface area
1.11 Place plates/flasks into a 37°C incubator overnight. Plates/flasks can remain for longer than one day, however they may dry out
1.12 Immediately prior to plating of inactivated MEF (inactivated by either radiation or mitomycin-C treatment), aspirate remaining gelatin solution. Either freshly inactivated MEFs or frozen inactivated MEFs can be used
1.13 Plate 2×10^6 inactivated MEF per 25 cm^2 flask

Cell culture of mouse ES cells

Note
The culture conditions may vary depending on the cell line used. Please refer to more detailed protocols cited under additional information before starting experiments.

1.14 The medium should be replaced every day!

1.15 Mouse ES cells should be passaged every second day and seeded on on freshly plated feeder cells. We recommend a subcultivation ratio of 1 : 4 to 1 : 10 (1×10^6 mouse ES cells per 25 cm^2 flask)

Trypsinization

1.16 24 hours before passaging the mouse ES cells plate inactivated MEF feeder cells on a gelatine-coated flask (2×10^6 inactivated MEF per 25 cm^2 flask)

1.17 Detach the mouse ES cells from the plate by trypsinization

1.18 Stop trypsinization by adding 4-fold media

1.19 As MEF feeder cells are also detached by this treatment, purge the cell suspension into an untreated flask (cell-culture treated) for 1 hour. MEF feeder cells accompanying the mouse ES cells will attach to the surface of the flask during this time

1.20 Collect the supernatant containing the mouse ES cells and plate it into a gelatine coated flask with inactivated feeder cells

2. Nucleofection™

Note

Prepare a 96-well culture plate with feeder cells for post Nucleofection™ culturing of the transfected cells 24 hours before Nucleofection™. Therefore plate 6.3×10^4 inactivated feeder cells into each well of a gelatin coated 96-well plate (6×10^6 cells for a complete 96-well plate).

One Nucleofection™ sample contains

- 5×10^4 cells
- 0.4–0.8 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 primary cell HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution

2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to the HT Nucleofector™ manuals)

2.3 Select the appropriate 384-well HT Nucleofector™ program **CG-104-AA**

2.4 Prepare cell gelatin coated culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 152 µl* (see note at the end of this chapter) for one well of a 96-well plate precoated with gelatin and inactivated feeder 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 (40 µl per sample)

2.6 Prepare 0.4–0.8 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Prepare the required amount of mouse ES cells by trypzination as described in 1.18–1.22

2.8 After incubating the trypsinized cell suspension for 1 hour on uncoated cell culture flasks, collect the mouse ES cells from by centrifugation (125×g, 10 minutes, RT). Remove supernatant completely

2.9 Resuspend the cell pellet carefully in 20 µl room temperature HT 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 384-well Nucleocuvette™ plates

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

- Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least multi-channel pipette with suitable pipette tips. As leaving cells in HT 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 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without und air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.11 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position

2.12 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)

2.13 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

2.14 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 48 µl of resuspended cells to 152 µl pre-warmed media

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 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 8–16 hours

Additional information

Up-to-date list of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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References

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