BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Protocol for Mouse Embryonic Stem [ES] Cells

For 4D-Nucleofector™ X Unit—Transfection in suspension

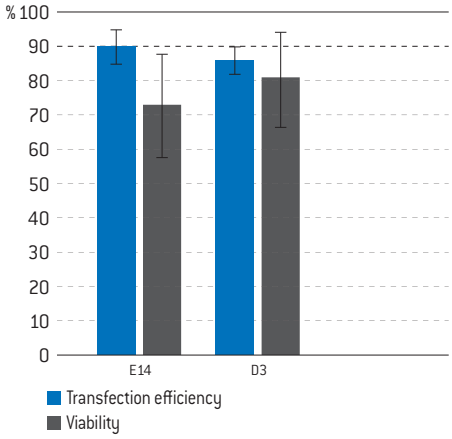
Cells derived from mouse blastocysts; round cells, growing in clumps

Notes

1. 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.
2. Experimental results and cell viability may vary depending on the mouse ES cell line used.

Example for Nucleofection™ of Mouse Embryonic Stem Cells

Average transfection efficiency of mouse ES cells 24 hours post Nucleofection™. 5 x 10^4 mouse ES cells were transfected with program CG-104 and 0.4 µg of pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ [Becton Dickinson]. Cell viability is given in percent compared to non-transfected control.



Product Description

Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032
Transfection volume	100 µl	100 µl	20 µl
Size [reaction]	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27 % overfill)	2.25 ml (1.968 ml + 13 % overfill)	0.675 ml (0.525 ml + 22 % overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27 % overfill)	0.5 ml (0.432 ml + 13 % overfill)	0.15 ml (0.115 ml + 22 % overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µl)	12	24	-
16-well Nucleocuvette™ Strips (20 µl)	-	-	2

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

4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. 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. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips:** 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 Instruments, 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 1µg/µl**

Note
For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- Cell 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, 100U/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)
- Prewarm appropriate volume of Mouse ES culture medium to 37 °C (see table 2)
- Appropriate number of cells/sample (see table 2)

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
1.2 Cells should be passaged 3 times per week with a subcultivation ratio of 1 : 3 to 1 : 10

Inactivation of MEF feeder cells

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

Preparation of gelatin coated plates

1.10 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
1.11 Autoclave for 30 minutes. Gelatin will solubilize and remain a liquid. Store at room temperature
1.12 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
1.13 Place plates/flasks into a 37 °C incubator overnight. Plates/flasks can remain for longer than one day, however they may dry out
1.14 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
1.15 Plate 2 x 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.

1.16 The medium should be replaced every day!
1.17 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 x 10^6 mouse ES cells per 25 cm^2 flask)

Trypsinization

1.18 24 hours before passaging the mouse ES cells plate inactivated MEF feeder cells on a gelatine-coated flask (2 x 10^6 inactivated MEF per 25 cm^2 flask)
1.19 Detach the mouse ES cells from the plate by trypsinization
1.20 Stop trypsinization by adding 4-fold media
1.21 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
1.22 Collect the supernatant containing the mouse ES cells and plate it into a gelatine coated flask with inactivated feeder cells

2. Nucleofection™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) 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 (see table 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
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,centrifuge the required number of cells (see table 3) at 125xg for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
2.10 Prepare mastermixes by dividing cell suspension according to number of substrates
2.11 Add required amount of substrates to each aliquot (max. 10 % of final sample volume)
2.12 Transfer mastermixes into the Nucleocuvette™ Vessels

Note
As leaving cells in 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.

2.13 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.14 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.15 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.16 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.17 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
2.18 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

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

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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References

1. Hogan, Constantini and Lacy, Manipulation of the mouse embryo,Cold Spring Harbor Laboratory Press
2. David A. Conner Harvard Medical School, Boston, Massachusetts
3. Current Protocols in Molecular Biology Copyright © 2003 John Wiley & Sons, Inc.
4. Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A. Smith, and Kevin Struhl (eds.)
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Table 1: Volumes required for a single reaction

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

Table 2: Required amounts of cells and media for Nucleofection™

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	12.5 ml	200 µl
Cell number per Nucleofection™ Sample	2–5 x 10^6 (Lower or higher cell numbers may influence transfection results)	5 x 10^4 (Lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection™ Sample and recommended program

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		2–5 x 10^6	5 x 10^4
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	2–20 µg	0.4–0.8 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		CG-104	CG-104

* Volume of substrate should comprise maximum 10 % of total reaction volume

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
10 cm culture dish	12 ml or appropriate volume	-
96-well culture plate	-	120 µl
Culture medium to be added to the sample post Nucleofection™	500 µl Mouse ES culture medium	80 µl Mouse ES culture medium

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	500 µl Mouse ES culture medium	80 µl Mouse ES culture medium
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	80 µl

* Maximum cuvette volume 200 µl