BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Optimization Protocol for Primary Cells or Cell Lines

For 4D-Nucleofector™ Y Unit—Transfection in Adherence

Cell Description

The optimization protocol enables you to optimize conditions for adherent Nucleofection™ of a primary cell or cell line of your choice in 24-well culture plates using our **Primary Cell Optimization 4D-Nucleofector™ Y AD kit** (V4YP-9A48).

To view an up-to-date list of all cells for which either an Optimized Protocol or customer data exist, refer to our on-line Cell Database www.lonza.com/celldatabase or contact our Scientific Support Team.

Product Description

Recommended Kit(s)

Primary Cell Optimization 4D-Nucleofector™ Y AD Kit

Cat. No	V4YP-9A48
Size (reactions)	48
AD1 4D-Nucleofector™ Y Solution	2 x 4.5 ml
AD2 4D-Nucleofector™ Y Solution	2 x 4.5 ml
Supplement	4 x 1 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	6 x 150 µg
Nunclon™ Δ Surface 24-well plate (Nunc)	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 of the Nucleofector™ Solution 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 be used only in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Optimization Guidelines

The initial optimization experiment should be performed with pmaxGFP™ Vector and is comprised of 24 reactions: Two 4D-Nucleofector™ Solutions (AD1 and AD2) are tested in combination with 11 different Nucleofector™ Programs and a "no program" control.

Day 0: Preparation and seeding of cells
Day 1: Adherent Nucleofection™
Day 2: Analysis

	AD1 Solution			AD2 Solution		
	1	2	3	4	5	6
A	CA-215	ED-142	EH-158	CA-215	ED-142	EH-158
B	CV-142	EG-142	FB-100	CV-142	EG-142	FB-100
C	DC-100	ER-137	FB-166	DC-100	ER-137	FB-166
D	DC-142	EW-166	No program control	DC-142	EW-166	No program control

No program = Addition of Nucleofector™ Solution and pmaxGFP™ Vector, but no application of program

The Nucleofection™ Condition with the highest efficiency and lowest mortality is selected for all subsequent experiments.

Optional: For receiving program suggestions for further fine-tuning of results, please submit your complete results to our Scientific Support Team.

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 (volumes required for a single reaction: 287 µl Nucleofector™ Solution and 63 µl Supplement).

- 4D-Nucleofector™ system (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ Y Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- 24-well Dipping Electrode Array compatible plates: Supplied Nunclon™ Δ Surface 24-well plate (Nunc). **Alternatively:** CELLSTAR™, 24W Plate [Greiner Bio-one, Cat. No. 662160]. Before using other types of 24-well plates for Nucleofection™, please contact Lonza Scientific Support to check if your 24-well plate is compatible with the Dipping Electrode Array
- **Optional:** Cover slip circles suited for 24-well-plates, max. height 0.25 mm [e.g. Menzel Gläser, Cat. No. CB00120RA1, 12 mm diameter, No. 1 (thickness of 0.13–0.16 mm)]
- Supplied pmaxGFP™ Vector
- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260: A280 ratio should be at least 1.8
- **For Coating:** If required, use your established coating substance and procedure. If you do not have an established protocol you may contact our Scientific Support Team
- **Culture medium:** For commercially available cells we recommend following the instructions of the supplier regarding culture medium and supplements
- Prewarm appropriate volume of culture medium to 37°C, 5% CO_2 (1 ml per sample)
- **Appropriate number of cells:** Depends on cell type and culture duration before Nucleofection™. In most cases cell numbers between 2.5 x 10^4 und 1 x 10^5 turned out to be appropriate when seeded 24 hours prior to Nucleofection™

1. Pre Nucleofection™

Coating of 24-well plates or cover clips

If any coating is required, use your established coating substance and procedure.

Cell culture recommendations

Note
For commercially available cells we recommend following the instructions of the supplier regarding media renewal, passaging and seeding conditions. Best Nucleofection™ results will be obtained with standardized cell culture conditions.

1.1 Cells should be plated for at least 24 hours before Nucleofection™
1.2 Cells should be 50–80% confluent at the time point of Nucleofection™

Transfer into 24-well culture plates

Note
Please make sure you are using the 24-well plate provided with the kit or another Dipping Electrode Array compatible plate (please see "Required Material")

1.3 Centrifuge the required number of cells (see "Required Material") at 80xg for 10 minutes at room temperature.
1.4 Resuspend the cell pellet carefully in an appropriate amount of pre-warmed cell culture medium (1 ml per sample)
1.5 Plate the desired amount of cells in the wells of the 24-well plate.
1.6 Incubate the cells in humidified 37°C/5% CO_2 incubator until Nucleofection™

2. Nucleofection™

One Nucleofection™ Sample contains

- Cells at 50–80% confluency
- 16 µg pmaxGFP™ Vector
- 350 µl AD1 or AD2 4D-Nucleofector™ Y Solution

Important notes:

(1) Nucleofection™ can be performed at any time during the culturing period

(2) Nucleofection™ performance may depend on cell species and type, isolation or thawing procedures, culturing and handling conditions and time point of Nucleofection™

(3) If cells were incubated for more than 4 days without changing media before Nucleofection™, it is recommended to wash cells twice with medium prior to Nucleofection™

(4) Please perform all pipetting steps very carefully to avoid disturbing adherence

(5) It is crucial that the cells are not permitted to dry out. In order to keep a small liquid film on the cells it is recommended to pipette off the medium or solution individually from each well. Usage of a vacuum pump is not recommended. Medium and solution removal and addition (see 2.6 and 2.12) should be performed carefully at the edge of the well

(6) Avoid air bubbles while pipetting

(7) Prevention of air bubbles underneath the 24-well Dipping Electrodes is important for the success of the Nucleofection™ Process. To reduce the accumulation of air bubbles underneath the electrodes during insertion of the 24-well Dipping Electrode Array into the plate, hold the 24-well plate in a 60–75° angle and insert the Dipping Electrode Array parallel to the surface of the 24-well plate (see figure below).

(8) Re-use of Dipping Electrode Array is not recommended and may lead to lower transfection efficiencies.

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: Please try all 11 recommended Nucleofector™ Programs (see Optimization Guideline) initially to determine the optimal one for your specific cell type

2.4 Pre-warm an aliquot of culture medium to 37°C (1 ml per sample)

2.5 Prepare 16 µg pmaxGFP™ Vector in 350 µl room temperature Nucleofector™ Solution per sample

2.6 Carefully remove media and immediately transfer 350 µl of substrate-solution mix into each well of the 24-well plate containing the cells (prepare each well individually, see note 5)

2.7 Insert the 24-well Dipping Electrode Array into the 24-well plate. Make sure that the Dipping Electrode Array is inserted in the right orientation (indicated by cut-off corners)

2.8 Place 24-well plate with inserted Dipping Electrode Array into the retainer of the Y-Unit. Well "A1" must be in upper left position

2.9 Start Nucleofection™ Process by pressing "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)

2.10 After run completion, carefully remove the 24-well plate from the retainer

2.11 Carefully withdraw the 24-well Dipping Electrode Array from 24-well plate without spilling liquid from one well to another and discard the array

2.12 Carefully remove Nucleofector™ Solution by pipetting and immediately add 1 ml of pre-warmed medium to each well (handle each well individually, see note 5)

3. Post Nucleofection™

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis

Additional Information

For an up-to-date list of all Nucleofection™ References, please refer to:

www.lonza.com/nucleofection-citations

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