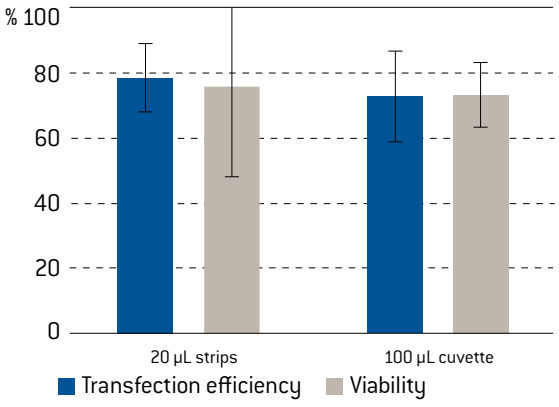
Bioscience Solutions

# 4D-Nucleofector™ Protocol for RBL-2H3 Cells

## For 4D-Nucleofector™ X Unit–Transfection in Suspension

Rat leukemia, basophil; fibroblastoid cells




### Example for Nucleofection of RBL-2H3 Cells

Transfection efficiency and viability of RBL-2H3 cells 24 hours post Nucleofection. RBL-2H3 cells were transfected with program DS-150 in 20 µL Nucleocuvette™ Strips (1 µg pmaxGFP™ Vector) or 100 µL Nucleocuvette™ Vessels (5 µg pmaxGFP™ Vector). 24 hours post Nucleofection, transfection efficiency was analyzed on a FACSCalibur™ (Becton Dickinson). Cell viability was determined using ViaLight™ Plus Assay and normalized to untransfected control sample.

## Product Description

**Recommended Kit(s) – SF Cell Line 4D-Nucleofector™ X Kit**

| Cat No. | V4XC-2012 | V4XC-2024 | V4XC-2032 |
|---|---|---|---|
| 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 conductive polymer Nucleocuvette™ Vessels, i.e. in the 4D-Nucleofector™ and the 96-well Shuttle™ System. They are not compatible with the Nucleofector™ II/2b Device.

## Required Material

**Note**
Please make sure that the supplement is added to the Nucleofector™ Solution prior to use. For preparing aliquots, mix Nucleofector™ Solution and Supplement in a ratio of 4.5 : 1 (see Table 1).

- 4D-Nucleofector™ System (4D-Nucleofector™ Core and 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 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**
When using pmaxGFP™ Vector as positive control, dilute the stock solution to an appropriate working concentration that allows pipetting of the recommended amounts per sample (see Table 3). Make sure that the volume of substrate solution added to each sample does 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
- **Culture medium:** Eagle's Minimal Essential medium with Earle's BSS with non-essential amino acids and sodium pyruvate [Lonza; Cat. No. 12-662F] supplemented with 15% fetal calf serum (FCS), 100 µg/mL streptomycin, 100 U/mL Penicillin and 2 mM UltraGlutamine I [Lonza; Cat. No. BE17-605E/U1] ], 1mM Na-Pyruvate [Lonza; Cat. No. BE13-115E], 0.1mM NEAA [Lonza; Cat. No. BE13-114E].
- Prewarm appropriate volume of culture medium to 37°C (see Table 2)
- Appropriate number of cells/sample (see Table 3)

## 1. Pre Nucleofection

### Cell culture recommendations

1.1 Passage cells every 2 – 3 days. A subcultivation ratio of 1:5 to 1:10 is recommended. Use low spin centrifugation (90xg)
1.2 Cells should not be used for Nucleofection™ after passage number 30
1.3 Optimal confluency for Nucleofection: 90%

### Trypsinization

1.4 Remove media from the cultured cells and wash cells once with an appropriate volume of PBS
1.5 For harvesting, incubate the cells ~5 minutes at 37°C with an appropriate volume of indicated trypsinization reagent (please see required material)
1.6 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5% BSA once the majority of the cells (>90% ) have been detached

## 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 Harvest the cells by trypsinization (please see 1.4 – 1.6)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see Table 3) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see Table 3)
2.11 Prepare mastermixes by dividing cell suspension according to number of substrates
2.12 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.13 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

2.14 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.15 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.16 Start Nucleofection Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.17 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.18 Resuspend cells with pre-warmed medium (for recommended volumes see Table 2). 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
2.19 Plate desired amount of cells in culture system of your choice (for recommended volumes see Table 2).

## 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

**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 plate format | | 6-well plate | 96-well plate |
| Culture medium | Pre-filled in plate | 1000 µL | 150 µL |
| | Added to sample post Nucleofection | 400 µL | 80 µL |
| Volume of sample transferred to culture plate | | complete sample (use supplied pipettes) | 50 µL |
| Final culture volume | | 1500 µL | 200 µL |

**Table 3: Contents of one Nucleofection sample and recommended program**

| | | 100 µL Single Nucleocuvette™ | 20 µL Nucleocuvette™ Strip |
|---|---|---|---|
| Cells | | $1 \times 10^6$ (Lower or higher cell numbers may influence transfection results) | $2 \times 10^5$ (Lower or higher cell numbers may influence transfection results) |
| Substrate* | pmaxGFP™ Vector | 5 µg | 1 µg |
| or | plasmid DNA (in $H_2O$ or TE) | 2–5 µg | 0.4–1 µg |
| or | siRNA | 30–300 nM siRNA (3–30 pmol/sample | 30–300 nM siRNA (0.6–6 pmol/sample) |
| SF 4D-Nucleofector™ X Solution | | 100 µL | 20 µL |
| Program | | DS-150 | DS-150 |

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

## Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to:
www.lonza.com/nucleofection-citations

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