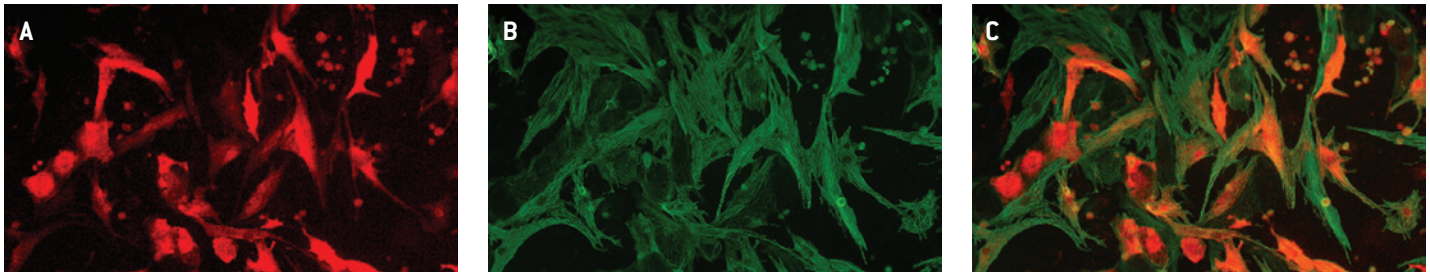
Amaxa® Rat Cardiomyocyte – Neonatal Nucleofector® Kit

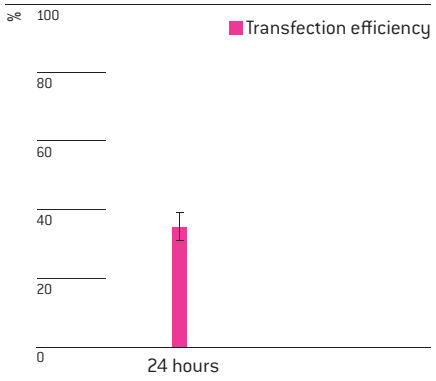
For Primary Rat Cardiomyocytes – Neonatal

Freshly isolated form newborn rats (Wistar rats); adherent cells with fibroblastoid morphology, cells contract ("beat") when grown at the required density

Example for Nucleofection® of neonatal rat cardiomyocytes



Primary neonatal rat cardiomyocytes were transfected using the Rat Cardiomyocyte-Neonatal Nucleofector® Kit program G-009 and 4 µg of a plasmid encoding DsRed [Clontech]. 2 days post Nucleofection®, cells were analyzed by fluorescence microscopy. Fig. A shows DsRed expressing cells. Cardiomyocytes stained with FITC-labeled tropomyosin antibody are shown in Fig. B. Fig. C shows a merge of Fig. A and B. (Photograph courtesy of F. Engel and M. Keating, Cardiology Department, Childrens' Hospital, Havard Medical School, Boston, MA, USA).



Transfection efficiency of primary neonatal rat cardiomyocytes 48 hours post Nucleofection®. Cells were transfected with program G-009 and 2.5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cell viability is usually 50-60%.

Product Description

Cat. No.	**VPE-1002**
Size (Reactions)	25
Rat Cardiomyocyte - Neonatal Nucleofector® Solution	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
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.

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. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- 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 of culture dishes**: 1% gelatine [Sigma; Cat. No. G-1890] solution in bidest water sterilized by autoclaving
- Gelatine coated 6-well culture dish or culture system of your choice
- **HEPES buffer (for cell preparation):** 20 mM HEPES-NaOH pH 7.6 [Sigma; Cat. No. H-3784], 130 mM NaCl [Sigma; Cat. No. S-5886], 3 mM KCl [Sigma; Cat. No. P-5405], 1 mM NaH_2PO_4 [Sigma; Cat. No. S-5011], 4 mM glucose [Sigma; Cat. No. G- 7021]. Use sterile-filtered or autoclaved salt stock solutions. Store sterile-filtered glucose stock solution at -20°C
- **Digestion buffer (for cell preparation)**: To 120 ml HEPES buffer (sufficient for 50 neonatal hearts), freshly add 16.8 mg collagenase type II [Invitrogen; Cat. No. 17101015], 48 mg pancreatin [Sigma; Cat. No. P-3292] and 1.8 mg DNase I [Roche; Cat. No. 104159]. Sterile filter under a laminar flow
- **Adhesion medium (for cell preparation)**: DMEM/F12 medium supplemented with 10% FBS, 2 mM glutamine and penicillin/streptomycin
- **Culture medium:** DMEM/F12 medium supplemented with 5% horse serum, 0.2% bovine serum albumine [Sigma; Cat. No. A-4161; ideally added from a stock solution, not solid], 2 mM L-glutamine [Lonza; Cat. No. 17-605E], 3 mM sodium pyruvate [Sigma; Cat. No. S-8636], 0.1 mM sodium L-ascorbate [Sigma; Cat. No. A-4034], 1 µg/ml insulin, 1 µg/ml transferrin, 10 ng/ml sodium selenite [Invitrogen; Cat. No. 51300-044], 0.02 mM cytosine-D-arabinofuranoside [Sigma; Cat. No. C-1768], 0.1 U/ml penicillin, 100 mg/ml streptomycin
- **Recovery medium** (optional, see note after step 2.10): RPMI 1640 supplemented with 10% horse serum
- Prewarm appropriate volume of culture media at 37°C (1.5 ml per sample)
- Appropriate number of cells (2 x 10^6 cells per sample)
  Minimal cell number: 8 x 10^5 (a lower cell number may lead to major increase in cell mortality)
  Maximum cell number: 4 x 10^6

1. Pre Nucleofection®

Note **Transfection results may be donor-dependent.**

Preparation of gelatine coated culture dishes

1.1 Incubate culture dishes or cover slips with sufficient amount of 1% gelatine solution for at least 2 hours at 37°C. Remove solution and let dry, ideally under germicidal UV light

Preparation of primary neonatal rat cardiomyocytes

Note **The isolation and culture of cardiomyocytes requires experience. It is essential for good cell viability to complete the isolation and transfection procedure as fast as possible. Please never resuspend cells by pipetting - simply tap the tube to resuspend the cell pellet. Further use 1000 µl pipette tips or larger for transferring the cells, even for 100 µl volume, to avoid damage by shear forces.**

1.2 Every medium, HEPES buffer and PBS should be pre-warmed to 37°C. Treating cardiomyocytes with cold solutions will impair their functionality. You also need an ice-cold aliquot of PBS

1.3 Sacrifice 2 – 3 day old rats and excise hearts from all pups. Store the excised hearts in calcium and magnesium-free PBS on ice

1.4 Squeeze hearts gently with forceps to expel the blood from the lumen. Transfer hearts into fresh ice-cold PBS

1.5 Move ventricles to a dry 6 cm Petri dish and mince tissue as small as possible with a scalpel blade

1.6 Transfer minced neonatal heart tissue into 20 ml of warm digestion buffer in a falcon tube and incubate for 5 minutes in a 37°C water bath. Mix either with a micro stirrer or by gentle shaking

1.7 Let the cells settle for 5 minutes, remove supernatant, add new pre-warmed digestion buffer and repeat enzyme treatment 6 – 7 times

1.8 Let cells settle down and wash once with pre-warmed HEPES buffer containing 5% horse serum. Eventually, spin cells down for 1 minute at 80xg

1.9 **A.** Resuspend cells in adhesion medium (20 ml per 10 hearts) and plate suspension on uncoated 10 cm dishes. Incubate for 1 – 1.5 hours at 37°C/5% CO_2. Repeat this step. During this time, fibroblasts will stick and spread on the plate whereas cardiomyocytes remain free

B. Alternatively, purify cardiomyocytes by Percoll or Ficoll gradient centrifugation. Percoll or Ficoll should be used at room temperature. We recommend using the adhesion step as Percoll or Ficoll may alter the cellular membrane characteristics of cardiomyocytes

1.10 Collect and count the cells to determine cell density. You may expect a yield of 0.8 – 1 x 10^6 cardiomyocytes per heart

1.11 Store cells in warm medium or buffer, if necessary. However, we recommend to continue right away with Nucleofection®

Note **Please follow this protocol as closely as possible. Otherwise, you will not be able to reproduce the transfection efficiencies and cell viabilities as indicated.**

2. Nucleofection®

One Nucleofection® sample contains

2 x 10^6 cells
1 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Rat Cardiomyocyte Nucleofector® Solution

Note **Please transfect freshly isolated cells only.**

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare gelatine-coated 6-well plates by filling appropriate number of wells with 2 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator

Note **We strongly recommend not to do more than three Nucleofection® Experiments at once. Storage of cells in Nucleofector® Solution will lead to increased cell mortality. Intermediately, remaining cells should be stored in medium or PBS at 37°C.**

2.3 Centrifuge the required number of cells (**2 x 10^6 cells per sample**) at **80xg for 1 minute** at room temperature
2.4 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
2.5 Combine 100 µl of cell suspension with **1 – 5 µg** DNA, 2 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates; 1 – 2 µg DNA are recommended for higher cell survival, 3 – 5 µg DNA for higher expression level
2.6 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
2.7 Select the appropriate Nucleofector® Program **G-009 (G-09** for Nucleofector® I Device**)**
2.8 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.9 Take the cuvette out of the holder once the program is finished
2.10 Add ~500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the prepared gelatine coated 6-well plate (final volume 2 ml media per well/sample). Use the supplied pipettes and avoid repeated aspiration of the sample

Note **In some cases it has proven advantageous to purge the cells off the cuvette with warm recovery medium. The cells are then plated into the gelatine coated 6-well plates containing the standard culture medium.**

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours but should preferably be analyzed at different times

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. R. von Harsdorf et al., Circulation 99 (1999) 2934-2941.
2. M.W. Bergmann et al., J. Mol. Cell Cardiol. 33 (2001) 1223-1232.

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