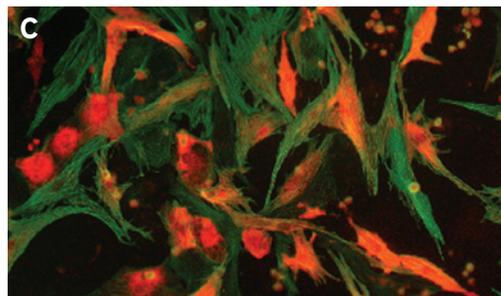
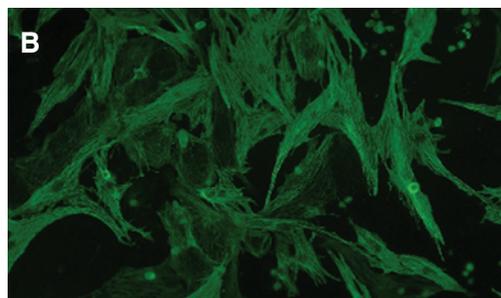
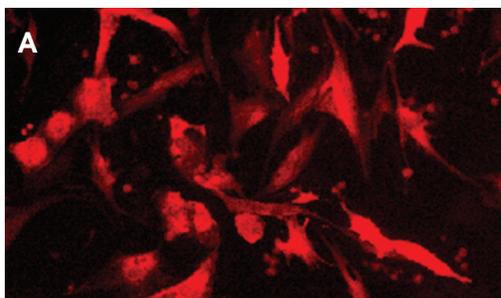


Rat Cardiomyocyte - Neonatal Nucleofector® Kit

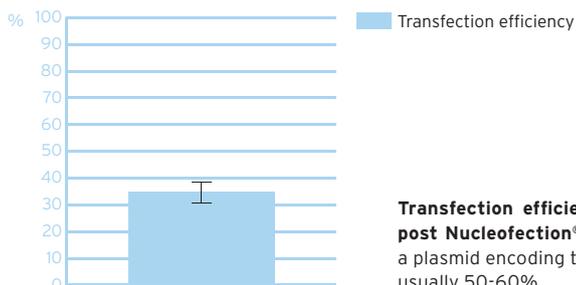
Primary Rat Cardiomyocytes - Neonatal

freshly isolated from 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, Harvard 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

- 2.25 ml Rat Cardiomyocyte - Neonatal Nucleofector® Solution
- 0.5 ml Supplement
- 10 µg pmaxGFP® (0.5 µg/µl in 10 mM Tris pH 8.0)
- 25 certified cuvettes
- 25 plastic pipettes

Size

25 reactions

Storage and stability

Store Nucleofector® Solution, Supplement and pmaxGFP® at 4°C. For long term storage pmaxGFP® is ideally stored at -20°C. The expiry 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!

- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- 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 NaH₂PO₄ [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 buffer for cell preparation: DMEM/F12 medium [e.g. ATCC; Cat. No. 30-2006] supplemented with 10% FBS [ATCC; Cat. No. 30-2020], 2 mM glutamine and penicillin/streptomycin
- Appropriate volume of culture media at 37°C (2 ml per sample); DMEM/F12 medium [e.g. ATCC; Cat. No. 30-2006] supplemented with 5% horse serum [PAA Laboratories; Cat. No. B15-021], 0.2% bovine serum albumine [Sigma; Cat. No. A-4161; ideally added from a stock solution, not solid], 2 mM L-glutamine [Invitrogen Cat.No. 25030-024], 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-arabino-furanoside [Sigma; Cat. No. C-1768], 0.1 U/ml penicillin, 100 mg/ml streptomycin
- Supplied pmaxGFP® control DNA
- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260:A280 ratio should be at least 1.8
- Nucleofector® device
- Appropriate number of cells (2 x 10⁶ cells per sample)
 - Minimal cell number: 8 x 10⁵ (a lower cell number may lead to major increase in cell mortality)
 - Maximum cell number: 4 x 10⁶

1. Pre Nucleofection®

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 !

Transfection results may be donor-dependent.

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 340xg
- 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% CO₂. 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 [Amersham Pharmacia]. 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 10⁶ 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®

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% CO₂ 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 10⁶ cells per sample**) at **340xg 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® DNA** or appropriate amount of **siRNA (30 nM-300 nM or 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

One Nucleofection® sample contains

- 2 x 10⁶ cells
- 1-5 µg plasmid DNA (in 1-5 µl H₂O or TE) or 2 µg pmaxGFP® or 30-300 nM siRNA (3-30 pmol/sample)
- 100 µl Nucleofector® Solution

2.7 Select the appropriate Nucleofector® program

G-009

- 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 RPMI 1640 medium containing 10% horse serum. The cells are then plated into the gelatine coated 6-well plates containing culture medium as described on page 2 (see required material).

3. Post Nucleofection®

- 3.1** Incubate the cells in a humidified 37°C/5% CO₂ 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

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.

For an up-to-date list of all Nucleofector® references, please refer to:

www.amaxa.com/citations

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