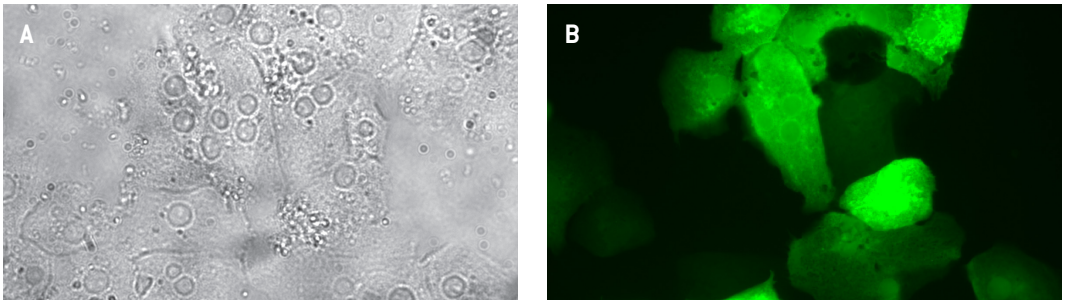
Amaxa® Rat Hepatocyte Nucleofector® Kit

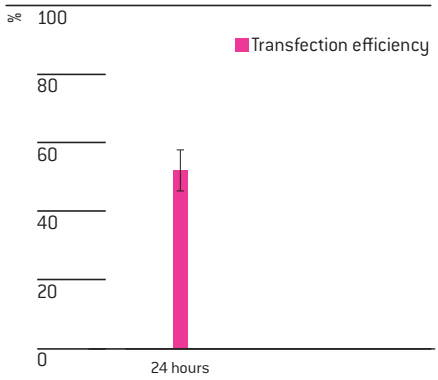
For Primary Rat Hepatocytes

Freshly-isolated; polygonal, adherent cells

Example for Nucleofection® of rat hepatocytes (Wistar) with maxGFP



Primary rat hepatocytes from Wistar rats were transfected with the Rat Hepatocyte Nucleofector® Kit, program Q-025 and a plasmid encoding maxGFP® Reporter Protein. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of rat hepatocytes. Primary rat hepatocytes from Wistar rats were transfected with program Q-025 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection®. Cell Viability (% trypan blue negative) is around 80% 24 hours post Nucleofection®.

Product Description

Cat. No.	**VPL-1003**
Size (reactions)	25
Rat Hepatocyte 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. 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; Software requirements: version **V1.9** or higher for Nucleofector I Device; version **S3.2** or higher for Nucleofector II 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
- 6-well culture dish or culture system of your choice coated with collagen type I [Sigma Cat. No. C-7661]; for preparation of dish please see 1.1-1.4
- **Isolation buffer 1**: 1l Krebs-Henseleit buffer (NaCl: 115 mmol/l; $NaHCO_3$: 25 mmol/l; KCl: 5.9 mmol/l; $MgCl_2$: 1.18 mmol/l; NaH_2PO_4: 1.23 mmol/l; Na_2SO_4: 1.2 mmol/l; $CaCl_2$: 1.25 mmol/l; glucose: 6 mmol/l)
- **Isolation buffer 2**: 1l Krebs-Henseleit buffer without Ca^{2+} and SO_4^{2-} (NaCl: 115 mmol/l; $NaHCO_3$: 25 mmol/l; KCl: 5.9 mmol/l; $MgCl_2$: 1.18 mmol/l; NaH_2PO_4: 1.23 mmol/l; glucose: 6 mmol/l)
- **Collagenase solution**: 150 ml of isolation buffer 2 plus 1.5 ml of a 10 mmol/l $CaCl_2$. solution plus 3 g of lyophilized bovine serum albumin plus 30 mg collagenase (e.g. Seromed, collagenase type CLS II)
- Preparation of 6 well plates for cultivation: 30% Ethanol; PBS
- **Culture medium**: William´s E, 100 U/ml Penicillin, 100 µg/ml Streptomycin, 5% FCS, 2 mM glutamine, 100 nM insulin, 100 nM Dexamethasone (from 1.5 mM stock solution in DMSO)
- Prewarm appropriate volume of culture medium to 37°C (2.5 ml per sample)
- Appropriate number of cells (4×10^5 – 4×10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Notes **(1) The isolation and culture of hepatocytes requires experience. Please make sure that these processes are established in your hands before you set out to transfect hepatocytes. (2) It is essential for good cell viability to complete the isolation and transfection procedures as fast as possible. Please make sure that all required reagents (medium, solutions, etc.) and materials (cuvettes, collagen-coated dishes, etc.) are ready for use before you sacrifice the animals and start the preparation. (3) Please transfect freshly isolated cells only. (4) Please pipette isolated cells as little as possible. (5) Preferrentially resuspend them by rolling the tubes.**

Preparation of 6 well plates for cultivation

1.1 To prepare a 2.5 mg/ml collagen stock solution, dissolve the collagen type I in 0.2% sterile acetic acid by stirring at room temperature for 4 hours

1.2 Add 20 µl collagen stock and 1 ml 30% ethanol per well and coat 6-well plates for 24 hours at room temperature under a laminar flow

1.3 Wash 2 x with PBS

1.4 Dry plates for about 15 minutes under a laminar flow

Isolation of cells:

1.5 Cool down centrifuge to 4°C
1.6 Rinse the perfusion setup extensively with distilled water
1.7 Thereafter rinse the perfusion setup with isolation buffer 2 at 37°C, gassed with carbogen (5% CO_2, 95% O_2)
1.8 Anesthetize a 6 week old rat with an intraperitoneal injection (e.g. ketanest 100 mg/kg body weight (BW) plus xylazin 5 mg/kg BW)
1.9 Place the animal on its back
1.10 Cut off the skin above the femoral vein and inject heparin (200 IE/ 100 g BW) into the femoral vein
1.11 Open the abdomen by an vertical incision up to the sternum
1.12 Push the small intestine towards the left side of the animal and push the liver upwards in order to expose the portal vein
1.13 Place loosely two clamps beneath the portal vein with curved tweezers
1.14 Check that the perfusion system is free of air bubbles. The system should be run with low flow (<5 ml/min)
1.15 Canulate the portal vein (16 gauge needle)
1.16 Fix the canula tightly with one of the clamps
1.17 Cut the aorta beneath the liver, cut the chest of the animal. Place a cut into the right atrium of the heart
1.18 Increase the flow rate according to the body weight: the flow rate should be 4 ml/g liver, the liver weight is approx. 4% of BW
1.19 Fix the canula in the portal vein with the second clamp
1.20 Ligate the vena cava beneath the liver with an additional clamp
1.21 Place a second canula of 14 gauge connected to a soft tube into the vena cava above the liver in order to enable a recirculating system. Fix the canula with a clamp
1.22 Perfuse the liver with isolation buffer 2 for 15 minutes, the temperature at the tip of the canula must be 37 to 38°C
1.23 Switch to collagenase solution, start in a non-recirculating mode until the perfusion system is completely filled with collagenase solution
1.24 Change to a recirculating perfusion mode with collagenase solution for another 15 minutes (the solution is collected via the canula/tube in the vena cava)
1.25 Take out the liver from the animal: cut the tissue between stomach/gut and the liver, cut off the liver from the diaphragm
1.26 Place the liver into a Petri dish together with the remaining collagenase solution
1.27 Disrupt the liver capsule (e.g. with the curved tweezers) and thoroughly shake the liver within the collagenase solution
1.28 Filter the cell suspension through gauze into a second Petri dish, dispense the suspension into centrifugation tubes (e.g. 50 ml tubes)
1.29 Centrifuge the suspension for 3 minutes at 25 to 50xg at 4°C (vital hepatocytes do have the highest sedimentation rate and will therefore preferentially sediment)
1.30 Discard the supernatants
1.31 Gently resuspend the cell pellet with cold isolation buffer 1 (should be gassed with carbogen). If cells stick together, they might be resuspended by very gentle pipetting with a 25 ml pipette. The cells should be resuspended in a volume of 30 to 50 ml (isolation buffer 1)
1.32 Filter the suspension through a cell strainer with a 70 µm mesh into new centrifugation tubes

1.33 Repeat steps 1.29 to 1.32
1.34 Centrifuge the suspension a third time for 3 minutes at 25 to 50 g at 4°C
1.35 After the third centrifugation resuspend the cell pellet in 20 ml of isolation buffer 1
1.36 Dilute an aliquot of the hepatocytes cell suspension 1 : 10 and count cells. You will get approximately 2 x 10^8 hepatocytes per liver
1.37 Determine viability e.g. by trypan blue staining (normally 90 to 95% of cells should be viable)
1.38 If required, dilute cells either with isolation buffer 1 or with hepatocyte culture medium
1.39 Cells should be transfected shortly after isolation (max. 1 hour storage on ice).

2. Nucleofection®

One Nucleofection® Sample contains

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

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare collagen-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
2.3 Count an aliquot of the cells and determine cell density
2.4 Centrifuge the required number of cells (**1 x 10^6 cells per sample**) at **25 – 50xg for 3 minutes at 4°C**. Remove supernatant completely
2.5 By rolling the tube, resuspend the cell pellet carefully to a final concentration of 1 x 10^6 cells/100 µl room-temperature Nucleofector® Solution per sample

Note **Avoid leaving the cells in Rat Hepatocyte Nucleofector® Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability and gene transfer efficiency.**

2.6 Combine 100 µl of cell suspension with **1 – 6 µg DNA**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM siRNA** (3 – 30 pmol/sample) or other substrates
2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close cuvette with the cap
2.8 Select the appropriate Nucleofector® Program **Q-025** (Q-25 for Nucleofector® Device I)
2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.10 Take the cuvette out of the holder once the program is finished
2.11 Incubate the sample in the cuvette for 15 minutes at room temperature
2.12 After the post nucleofection step, add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6-well plate (final volume 2.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 After 4 hours carefully replace medium with fresh culture medium (hepatocytes should be adherent at this time)

Additional Information

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

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