LabBOOK

VIROMER® BLUE
VIROMER® GREEN

siRNA/miRNA transfection

Contact

Bettina Weber (Biologist)

+49 345 55 59 625
bettina.weber@lipocalyx.de

Dr. Sandra Lagauzère (Biologist)

+49 345 55 59 663
sandra.lagauzere@lipocalyx.de

General information

Technology

Viromer® BLUE and **Viromer® GREEN** are polymer-based transfection reagents featuring a viral mechanism of membrane fusion. They do form transfection complexes with small interfering RNA (siRNA) or microRNA (miRNA) having a size of a few hundred nanometers. Viromer®: RNA complexes are taken up by endocytosis, a process that involves the formation of an acidic compartment. The low pH in late endosomes acts as a chemical switch that renders the Viromer® surface hydrophobic and facilitates membrane crossing. This "Active Endosome Escape" technology is safe and maximizes transfection efficiency as it is using a natural uptake pathway.

Key Benefits

+ Active Escape Technology	> Efficacy and safety during uptake
+ Zero Charge	> Fully compatible with serum or antibiotics. Fully compatible with suspension cells.
+ Stable Particles	> Reproducible results
+ Lipid free	> Works in adipocytes
+ Reverse Transfection	> Ready for High-Throughput Screening

Application

Viromer® BLUE and **Viromer® GREEN** are optimized for the *in vitro* transfection of siRNA and miRNA. They are highly effective on a wide range of standard and hard-to-transfect cells including suspension cells, stem cells and primary cells. For the transfection of plasmid DNA and mRNA, please refer to **Viromer® RED** and **Viromer® YELLOW**.

Viromer® BLUE or Viromer® GREEN?

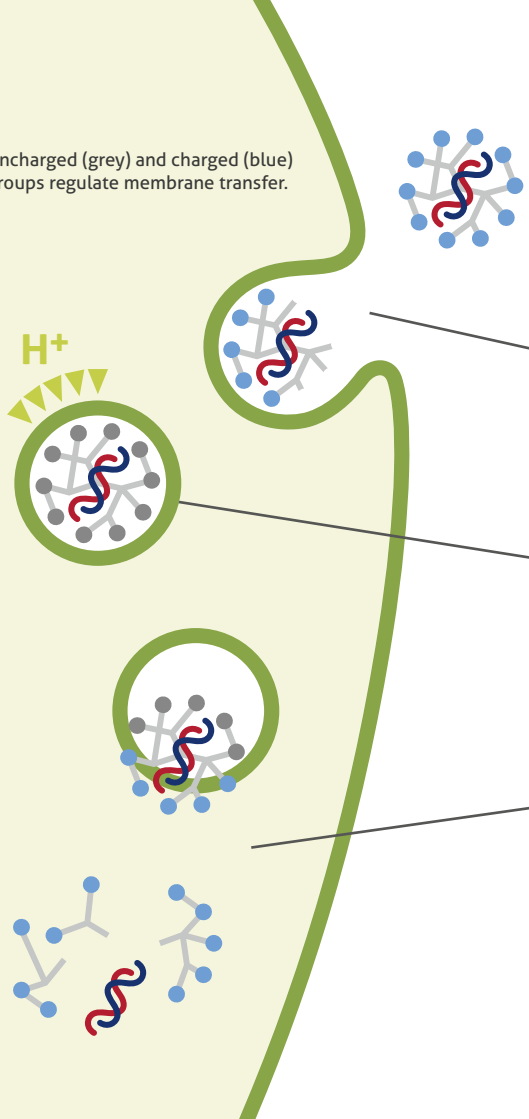
The two transfection reagents differ in their surface and backbone chemistry but follow the same workflow.

• **Viromer® BLUE**	> is a versatile standard reagent
• **Viromer® GREEN**	> is more selective for particular cells

A list of cell types and transfection results is available at:
https://viromer-transfection.com/data-by-cell-type/cell-transfection-a-z. If the cell type of your interest is not listed, parallel tests with both **Viromer® BLUE** and **Viromer® GREEN** are recommended.

The Viromer® Uptake Pathway: The Active Escape Process

Uncharged (grey) and charged (blue) groups regulate membrane transfer.



1:00h – early endosome

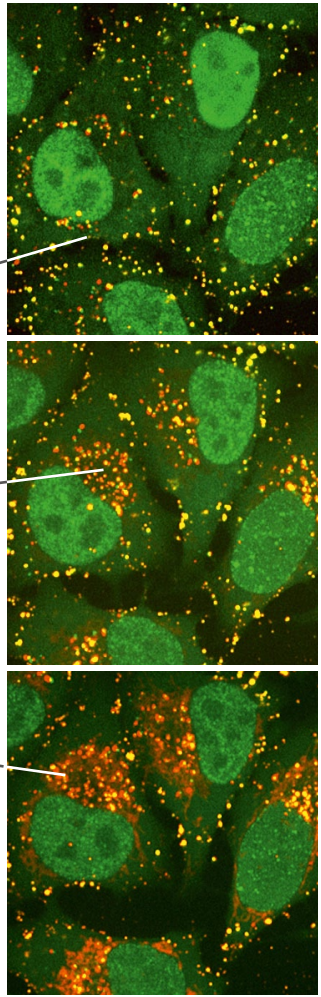
Viromers are taken up at the cell surface.

3:00h – late endosome

Viromers accumulate near the nucleus, ongoing acidification.

4:30h – arrival in the cytosol

Discharge of siRNA from endosomes and starting diffusion.



Model: HeLa, Viromer® GREEN, labelled siRNA. Data courtesy of Chromotek.

Protocol Guidelines

Material required

Use a sterile workplace and materials as required for any cell culture work. Warm all reagents to room temperature. Prepare fresh transfection complexes each time you use the products. **Buffer BLUE** and **Buffer GREEN** (provided with the kits) are required to make dilutions of Viromer® reagents and siRNA or miRNA.

Cell Culture and Plating

Viromer® **BLUE** and Viromer® **GREEN** are compatible with standard cell culture media, serum and antibiotics.

- Seed the cells in complete medium the day before transfection
- Adjust the cell density so that 60 - 80% confluency are reached at the time of transfection
- For experiments lasting for more than 48h, replenish medium before starting transfection.

Note: The preparation of suspension cells might also be achieved few hours before transfection.

Recommended number of cells to be seeded one day before transfection (for standard cells, read-out 24h - 48h post-transfection)

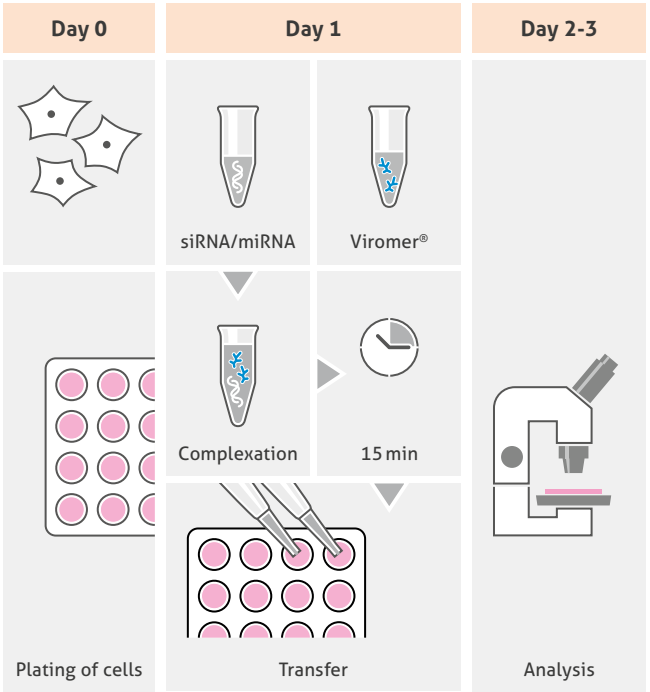
Multiwell plate type	96-well	24-well	6-well
Adherent cells			
Cells seeded per well	12,000	60,000	250,000
*Range	± 3,000	± 20,000	± 80,000
Suspension cells			
Cells seeded per well	48,000	240,000	1,000,000
*Range	± 12,000	± 80,000	± 320,000
Medium per well	0.1 ml	0.5 ml	2 ml

* In reverse transfection protocols, cell numbers should be on the higher end.

The optimum density for transfection is highly variable among cell types and needs to be determined empirically. Please refer to optimization on page 11 for more details.

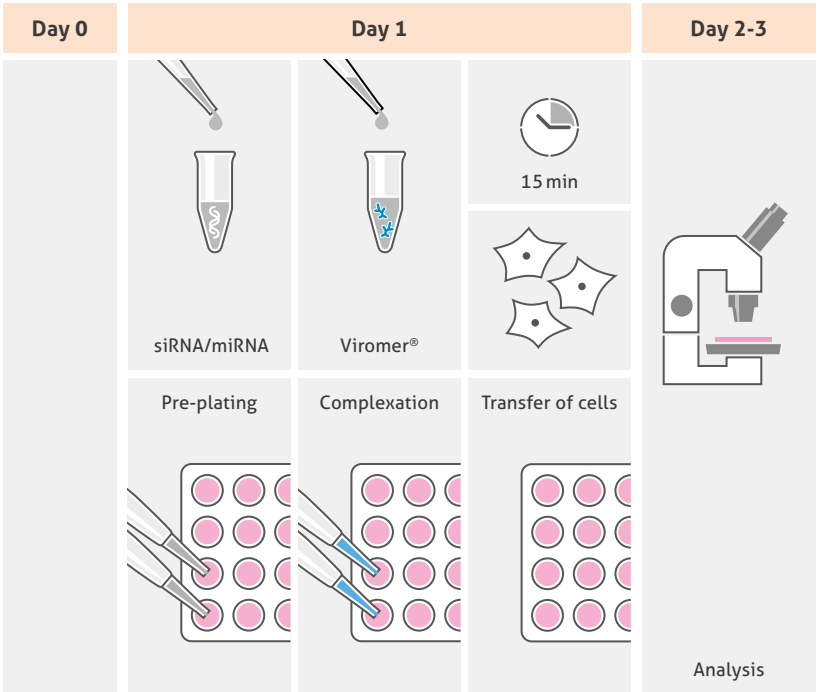
Transfection workflow

We here show a common workflow for the Forward Transfection of adherent or suspension cells using Viromer® **BLUE** and Viromer® **GREEN**.



For Reverse Transfection and high-throughput screening (HTS) applications, the use of Viromer® **BLUE** or Viromer® **GREEN** is based on a common protocol in which all steps - complexation, transfer in wells and adding of cells - are performed at the same day.

Please contact the Viromer® technical support for detailed information.



General Tips for Transfection at the bench!

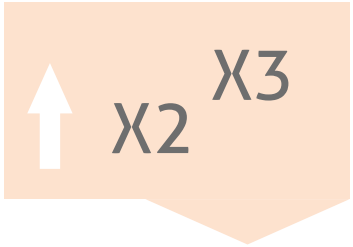
Add extra volume

All protocols given here are based on exact volumes for single-well transfections. Whenever possible, plan for larger experiments including replicates and prepare a master mix. Work with an extra 10% of all volumes to account for pipetting errors.

Use minimum volumes

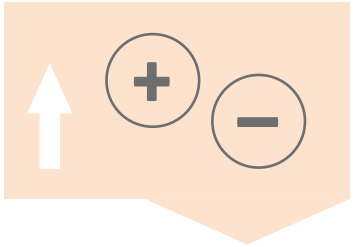
We advise to prepare at least 50µl of transfection complex to ensure homogenous complexation and to limit variability.

Replicates



As for any other bench work involving cells in culture or preparation of very small volumes, plan for replicates to create a solid base of data for the interpretation of results and related statistical analysis.

Controls



siRNA-mediated knock-downs should include:

- Untreated cells
- Positive control siRNA (targeting a reporter or housekeeping gene)
- Negative control siRNA (non-targeting)
- On-target siRNA for your gene of interest

Refer to Start Positive® Controls for a set of positive and negative controls.

Basic Transfection Protocol for **Adherent cells**

1 Preparation of siRNA » Tube 1

- Dilute siRNA in provided buffer to **2.75 µM**
- Prepare a volume of **5 µl**

2 Preparation of Viromer® » Tube 2

- Place a **0.5-µl** droplet of Viromer® onto the wall of a fresh tube
- Immediately add **45 µl** of buffer and vortex 3-5 s
  Always add buffer to Viromer®, not vice versa!

3 Complexation » Tube 2 –> Tube 1

- Pipette **45 µl** of the Viromer® solution from Tube 2 on the **5 µl** of siRNA solution in Tube 1
- Mix swiftly and incubate 15 min at room temperature

4 Add 50µl of the transfection complex on the cells

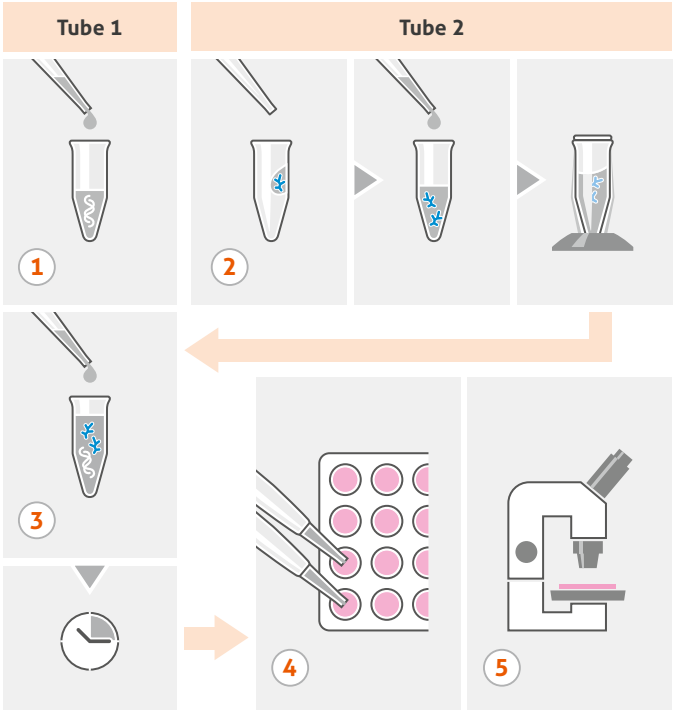
5 Read-out

- Incubate cells as usual. No need to change medium unless high amounts of transfection complex cause toxicity.
- Monitor effects 24-48 hours post-transfection at the mRNA level expression, or 48-96 hours post-transfection for proteins.

Corresponding volumes in other culture formats

Multiwell plate type	96-well	24-well	6-well
Tube 1	1 µl	5 µl	20 µl
Tube 2	0.1 µl Viromer® 9 µl buffer	0.5 µl Viromer® 45 µl buffer	2 µl Viromer® 180 µl buffer
Transfer volume	10 µl	50 µl	200 µl

24-well format | 25 nM final siRNA concentration



Note: All volumes are given as exact volumes for single-well transfections.

Basic Transfection Protocol for **Suspension cells**

1 Preparation of siRNA » Tube 1

- Dilute siRNA in provided buffer to **11 µM**
- Prepare a volume of **5 µl**

2 Preparation of Viromer® » Tube 2

- Place a **0.5-µl** droplet of Viromer® onto the wall of a fresh tube
- Immediately add **45 µl** of buffer and vortex 3-5 s
  Always add buffer to Viromer®, not vice versa!

3 Complexation » Tube 2 –> Tube 1

- Pipette **45 µl** of the Viromer® solution from Tube 2 on the **5 µl** of siRNA solution in Tube 1
- Mix swiftly and incubate 15 min at room temperature

4 Add 50 µl of the transfection complex on the cells

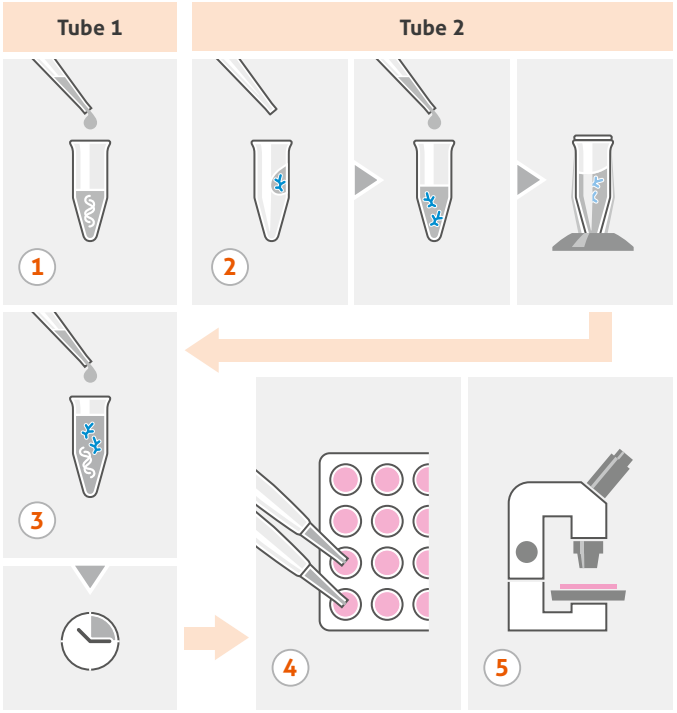
5 Read-out

- Incubate cells as usual. No need to change medium unless high amounts of transfection complex cause toxicity.
- Monitor effects 24-48 hours post-transfection at the mRNA level expression, or 48-96 hours post-transfection for proteins.

Corresponding volumes in other culture formats

Multiwell plate type	96-well	24-well	6-well
Tube 1	1 µl	5 µl	20 µl
Tube 2	0.1 µl Viromer® 9 µl buffer	0.5 µl Viromer® 45 µl buffer	2 µl Viromer® 180 µl buffer
Transfer volume	10 µl	50 µl	200 µl

24-well format | 100 nM final siRNA concentration



Note: All volumes are given as exact volumes for single-well transfections.

Transfection optimization

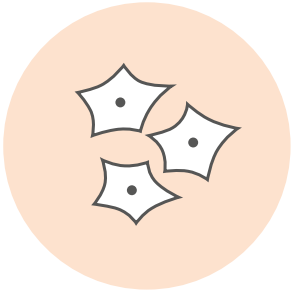
You do see efficacy, but your siRNA mediated knock-down is far from complete or your non-target control is close?

Except for cases where the basic protocol is absolutely right, we suggest reproducing the initial findings and creating stronger data with a few optimization steps.
Cell density, amount of transfection complex, Viromer®: siRNA ratio, and time point of the analysis are the key factors for an optimization. For any new combination of cells and siRNA sequences, we strongly recommend adjusting each parameter one by one using the following guidelines.

1. Amount of transfection complex

Test increasing amounts of transfection complex arriving onto the cells

Maximize siRNA effect using a fixed Viromer®: siRNA ratio



2. Cell density & confluency

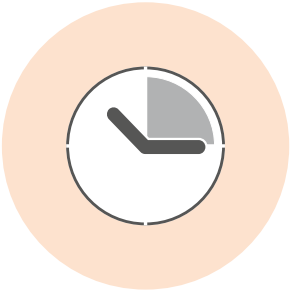
Growth, cell division or the existence of cell contacts regulate uptake of materials

Cell density is a strong regulator of siRNA effects

3. Viromer®: siRNA ratio

Higher concentration of Viromer® results in better transfection.

Maximize efficacy at low concentrations of siRNA



4. Time for analysis

24-72h depending on siRNA amount. Change of medium 4h post-transfection if toxicity is observed

The perfect timing for optimal read-outs

Amount of Viromer®: siRNA transfection complex

Five-step titration to determine the lowest siRNA amount (final concentration in well) that could be used.

1 Provide siRNA at 2.75 µM for adherent cells or 11 µM for suspension cells. Use provided buffer to make dilutions. — Tube 1

2

	96-well	24-well	6-well		
Viromer®	0.6 µl	3 µl	12 µl	Buffer on Viromer®, not vice versa!	Tube 2
Buffer	53.4 µl	267 µl	1068 µl		

3

Viromer® from step 2	54 µl	270 µl	1080 µl	Master mix for complexation (incubate for 15 min).	Tube 2 » Tube 1
siRNA from step 1	6 µl	30 µl	120 µl		

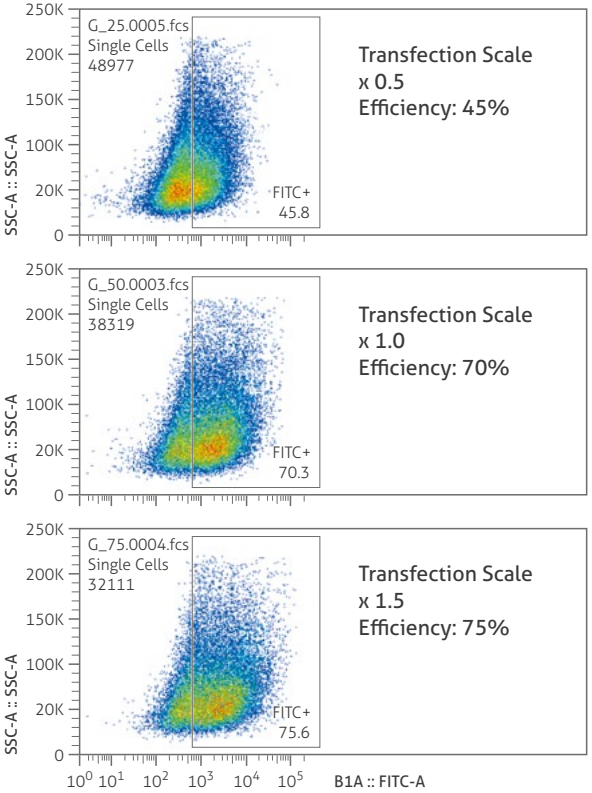
4

	Transfer volume per well			Final siRNA concentration	
Transfection Scale				Adherent	Suspension
0.2 x	2 µl	10 µl	40 µl	5 nM	20 nM
0.5 x	5 µl	25 µl	100 µl	12.5 nM	50 nM
1.0 x Standard	10 µl	50 µl	200 µl	25 nM	100 nM
1.5 x	15 µl	75 µl	300 µl	37.5 nM	150 nM
2 x	20 µl	100 µl	400 µl	50 nM	200 nM

5 Monitor siRNA effects 24 - 72 h after transfection.

Note: This protocol is designed for one siRNA sequence without replicates. Do parallel experiments for the respective controls.

Adherent cells: 5 - 50 nM final siRNA conc.
Suspension cells: 20 - 200 nM final siRNA conc.



Increasing efficiency of siRNA-mediated knock-down in MCF-10A cells transfected with **Viromer® BLUE** at three different transfection scales.

FACS data from E. Hadadi, ES Team-INSERM U935, Paris (France).

Cell density and confluency

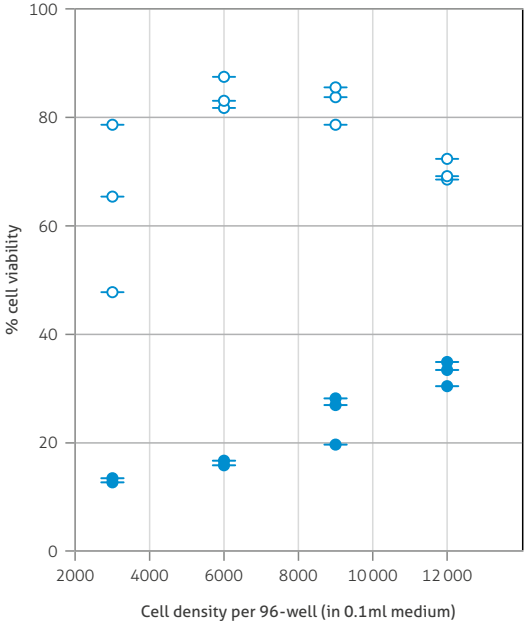
For each cell type, an optimum cell density for transfection needs to be determined.

› Target: **60-80%** at the time of transfection

› Usual recommendations for common cells: seed the cells one day prior transfection at
- **8-15 x 10^4 cells/ml for adherent cells,**
- **36-60 x 10^4 cells/ml for suspension cells**

› Take into account
- cell specifics for adjustments e.g. growth rate, size, cell junctions
- selected assays and time-point requirements e.g., for short-term assays, plate cells at higher density than for long-term assays.

› Once optimized, keep parameters constant to ensure reproducibility in further experiments



Optimum cell density for siRNA-based knock-down in HeLa cells.

Phenotypic assay on HeLa cells (PLK-1 siRNA stops cell cycle) plated one day prior transfection at 4 different densities (3, 6, 8 and 12 x 10^4/ml) - Basic standard protocol with **Viromer® BLUE**, read-out 72h post-transfection.

» Experiments become overly sensitive at very low density but loss of signal at high density. Optimum transfection efficacy was obtained for 6 x 10^4 cells/ml, which is lower than the recommended averaged range. Explained by high doubling rate and relative large size of HeLa cells in combination with late read-out (72h).

PLK-1 scr

Seed cells at ...

Recommended range
- Adherent cells: 8-15 x 10^4 cells/ml
- Suspension cells: 36-60 x 10^4 cells/ml

Start at lower density,

- Large cells
- High doubling rate
- Cells with tight junctions
- Read-out >48h

or high density.

- Small cells
- Low cell growth
- Difficulty to reach confluency
- Read-out <24-48h
- Reverse transfection

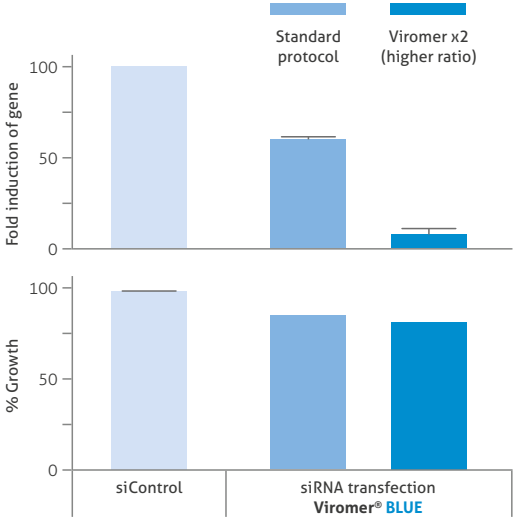
Analyse cells at ...

60–80% confluency

VIROMER®: siRNA ratio

If no toxicity is observed, transfection efficiency can be enhanced by using more Viromer® reagent for a fixed amount of siRNA. We suggest adding more Viromer® reagent (x2, x2.5, x3...) at the dilution step (step 2 of the protocol) while keeping all other volumes constant.Inversely, if transfection at standard conditions is toxic to the cells, a lower ratio should be tested.

BV-2: Mouse Microglia-Like Cells

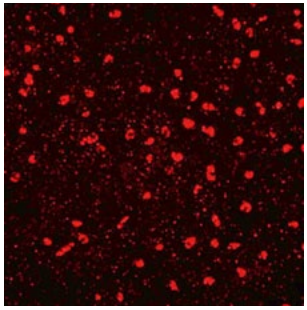


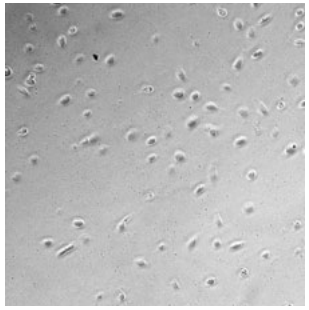
Data from V. Mathur, T. Wyss-Coray's Lab, Standford University, Palo Alto, USA

Time analysis

Maximum effects of siRNA are usually observed 24-48 hours when analyzed at the mRNA level. The reduction of the respective protein needs additional time and should be monitored between 48 and 96h post transfection.
It is recommended to change the medium 4h after transfection in case of toxicity to the cells. Optionally, supplementation or replacement of medium can be done 24h or 48h after transfection.

Rat Primary Microglia cells





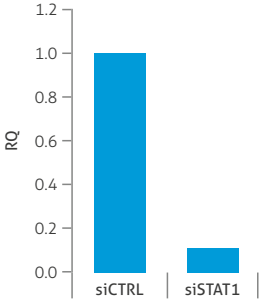
Transfection of 3 siRNA (red-labeled GLO, STAT1 and STAT3) with **Viromer® BLUE**

Up: Microscopic observations of siGLO-red in cells 48h-post transfection;

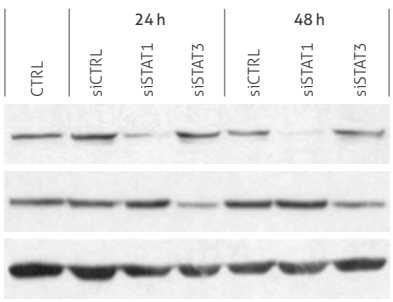
bottom left: silencing of STAT1 gene at the mRNA level 24h post-transfection;

bottom right: silencing of STAT1 and STAT3 genes at the protein level, 24h- and 48h-post transfection.

RT-PCR



Western Blot



"We are very satisfied with the results. We see good silencing both at the mRNA and protein levels. Cells are viable in 80-90% under the condition that we change medium after 4h."

M. Maleszewska,
Nencki Institute of Experimental Biology
Warsaw, Poland

Towards 100% knock-down (dose-response curve)

As a final step in the optimization, the siRNA concentration should be limited to the amount necessary to obtain a clear phenotype with maximum separation from any response to a control siRNA. **In such dose-response experiments, a fixed amount of Viromer® is used while only the siRNA concentration varies.** Practically, provide serial dilutions of your siRNA and perform parallel transfections.

The protocol below is using the standard conditions for adherent cells in 24-well. Please, adapt to previously optimized parameters. To model the regression curve, use at least 3 replicates for each condition.

1 Preparation of siRNA » 6x Tube 1

- Prepare serial dilutions of siRNA starting at **11µM** in buffer.
- Provide volumes of **5µl**

Final siRNA on cells [nM]	100	50	**25**	12.5	6.25	3.125
Aliquot solutions [µM]	11	5.5	**2.75**	1.325	0.66	0.33

2 Preparation of Viromer® » Tube 2

- Place a **3.5µl** droplet of Viromer® onto the wall of a fresh tube
- Immediately add **311.5µl** of buffer and vortex 3-5s
  Always add buffer to Viromer®, not vice versa!

3 Complexation » Tube 2 –> Tube 1

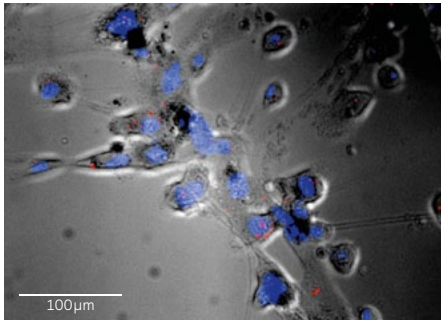
- Pipette **45µl** of the Viromer® solution from Tube 2 on the **5µl** of siRNA solution in Tube 1, and mix swiftly
- Repeat procedure with all other tubes
- Incubate 15min at room temperature

4 Add 50µl of the transfection complex on the cells

5 Read-out

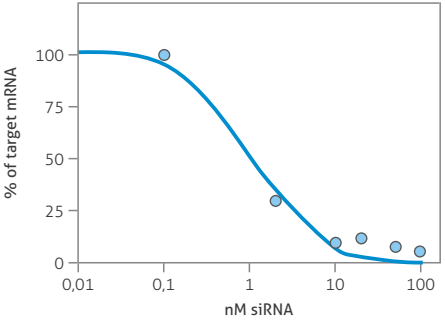
- Incubate cells as usual.
- Monitor effects 24-48 hours post-transfection at the mRNA level expression, or 48-96 hours post-transfection for proteins.

Primary Human Skeletal Myoblasts



Microscopic observation of human primary myoblasts after transfection with a Cy3-labeled siRNA using **Viromer® BLUE**.

Data generated by J. A. Zagalak, ETH Zürich – IPW, Switzerland



Data generated by C. Weigert, University of Tübingen, Germany

Trouble-shooting / Minimizing Background

Low transfection efficiency or no signal

First, follow recommendations for protocol optimization given previously:

- Use higher amount of transfection complex onto the cells, either by increasing the final siRNA concentration or by seeding less cells per well
- Use higher amount of Viromer® in step 2 of the protocol
- Monitor effects at various time points from 24h to 96h post-transfection
- Ensure that the cell density is adequate

Additional tips:

- Check that Viromer® reagents were stored and used correctly.
- Transfection complexes should be prepared freshly.
- All materials should be sterile and RNase free.
- Control that siRNA sequences are high-quality and ensure concentrations are correct.

Cell toxicity

- Change the medium 4h after transfection
- Use a lower amount of transfection complex on your cells
- Use less Viromer® in step 2 of the protocol
- Consider using a different siRNA. Keep in mind that even non-target control sequences may create background.
- Finally, ensure that silencing of the target gene does not induce cytotoxicity, e.g. by blocking vital pathways.

Type of siRNA

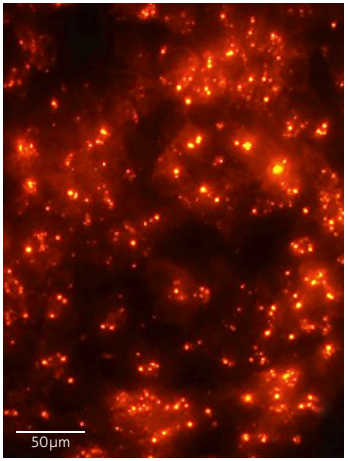
siRNA designs have seen major updates to improve the specificity and reduce immunogenicity. We recommend using siRNA pools and chemically modified siRNAs. If immunogenicity is a concern, monitor levels of central genes coding for essential proteins, such as OAS1.

Use the Viromer® Start Positive® Controls!

Positive® Controls are pre-formulated Viromer® transfection complexes. Use these materials for evaluating transfection of new cell types with the Viromer® technology or as reference material.

One kit of Start Positive® Controls comprises:

- a GAPDH-siRNA complexed to the Viromer® reagent
- a non-targeted siRNA labeled with Cy3 complexed to the Viromer® reagent



Monitoring of transfection complex uptake by MCF-7 cells transfected with a Cy3-labeled siRNA using **Viromer® BLUE Start Positive® Controls** (fluorescence microscopy).

Internal data Lipocalyx GmbH

Product information

Applications

Viromer® BLUE and **Viromer® GREEN** are optimized for *in vitro* transfection of siRNA and miRNA.

Content and formats

Viromer® BLUE Incl. Buffer BLUE	100 transfections	VB-01LB-00
	600 transfections	VB-01LB-01
	3 x 600 transfections	VB-01LB-03
Viromer® GREEN Incl. Buffer GREEN	100 transfections	VG-01LB-00
	600 transfections	VG-01LB-01
	3 x 600 transfections	VG-01LB-03

In standard conditions (no optimization), **0.5µl of Viromer® is sufficient for 1 reaction of transfection in the 24-well plate format.** Standard size packs have 300µl of concentrated Viromer® and 50ml of buffer.

Buffer BLUE and Buffer GREEN (pH 7.2 aqueous solutions) are required for diluting the Viromer® reagents and siRNA or miRNA.

Storage and use

Viromer® BLUE and **Viromer® GREEN** should be stored at **+2-8°C** in the provided aluminum bags.
They are then stable for 6 months (#VB-01LB-00/#VG-01LB-00) to **1 year** (#VB-01LB-01/#VB-01LB-03/#VG-01LB-01/#VG-01LB-03). As the reagents are sensitive to atmospheric CO_2, it is recommended to always close the vials and tighten the caps immediately after use. Avoid contact of the reagents with dry ice.

Quality control

Each batch of Viromer® is tested for transfection using a PLK-1 and control siRNA. Buffer solutions are analyzed for composition, sterility and RNase/DNase activity. MSDS are available at www.viromer-transfection.com

Product use limitations

These products are intended **for research use only**; they must not be used for therapeutic, veterinary or diagnostic applications. The purchase of Viromer® reagents implies a limited, non-transferable right to the purchaser to use these products, or parts from these products, only for its internal research. All further commercial applications of Viromer® products require a license from Lipocalyx GmbH.

www.viromer-transfection.com

Lipocalyx GmbH | Weinbergweg 23 | 06120 Halle | Germany

VIROMER® **BLUE**

VIROMER® **GREEN**

2017