Viromer®
BLUE, GREEN and BLACK

In vitro **siRNA** Standard Transfection Protocol

Product Information

This package contains Viromer BLUE, GREEN or BLACK; three new, functionalized transfectants specifically developed for siRNA / miRNA transfections. Viromers are polymers and do not contain lipids.

Technology: Viromers® take advantage of a viral fusion mechanism (hence their name) and translate this into polymer chemistry. The "membrane-like" character is provided by alkyl moieties in combination with long chain fatty acids. During endocytosis, Viromers will become exposed to an acidic environment. The low pH renders the fatty acid moieties uncharged and hydrophobic, a switch that facilitates membrane crossing.

Key Benefits:

Active Endosome Escape	Maximizes transfection efficacy and reduces background
Zero Charge	No need to remove serum or antibiotics Gentle on cells
Stable Particles	Lead to reliable and reproducible results
Lipid-free	No interaction with cell metabolism in particular with lipid metabolism
Reverse Transfection	Ready for High-throughput Screening

BLUE, GREEN or BLACK?

What is different?

BLUE, GREEN and BLACK differ in their surface or backbone chemistry.

Why testing more than one?

While we have optimized the active endosome escape, Viromers have no built-in cell specific motifs. Hence their uptake may differ between cell types, a feature that is hard to predict.

Selection guidance

BLUE, GREEN and BLACK all work on standard cell lines including many of the typical adherent cancer cells that are frequently used in cell biology labs. Cell preferences are noted, though, on more specialized cell types such as primary cells, suspension cells or stem cells.

BLUE has established itself as a versatile standard with broad support in the user data.
GREEN is more selective for particular cells.
BLACK is our latest product that was freshly optimized from a 10k Viromer library and has excellent performance and safety data.

Please see our reference table on our website for cell preference information based on user data. For a full optimization, do parallel tests with all Viromers.

www.lipocalyx.de
RNAi data

Content: Package contains 300µl of Viromer® BLUE, GREEN or BLACK and an appropriate amount of Buffer F, pH7.2. Free samples contain 50µl Viromer.

Reagent use: The product is optimized for the transfection of siRNA.
For the transfection of plasmid DNA, please refer to Viromer® RED or YELLOW.

Quality control: Each batch of Viromer® BLUE, GREEN or BLACK is tested for transfection using a PLK-1 and control siRNA. Buffer F was analyzed for composition, sterility and RNAse activity. MSDS are available at www.lipocalyx.de.

Storage: Viromers® should be stored between 2-8°C and are stable for 1 year. Please always tighten the screw caps tube to avoid evaporation or contamination.

Product use limitations: This product is intended for research use only; it must not be used for therapeutic, veterinary or diagnostic applications. The purchase of this product implies a limited, non-transferable right to the purchaser to use this product, or parts from this product, only for its internal research. All further commercial applications of Lipocalyx products require a license from Lipocalyx GmbH.

Before You Start

Cell culture: Viromers® can be used in forward or reverse transfection and work also with suspension cells.
In forward transfection protocols, cells are seeded the day before transfection and the Viromer:siRNA complexes are freshly prepared at the transfection day. Recommended starting conditions for common cell culture plates are:

Multiwell plate type	**96**	**48**	**24**	**12**	**6**
Adherent cells seeded per well	12,000 ±3,000	30,000 ±10,000	60,000 ±20,000	125,000 ±40,000	250,000 ±80,000
Medium per well (ml)	0.1	0.25	0.5	1	2
Typical volume of transfection complex (µl)	10	25	50	100	200

In reverse transfection protocols, cell numbers should be on the higher end, for suspension cells use 4x the numbers provided above.

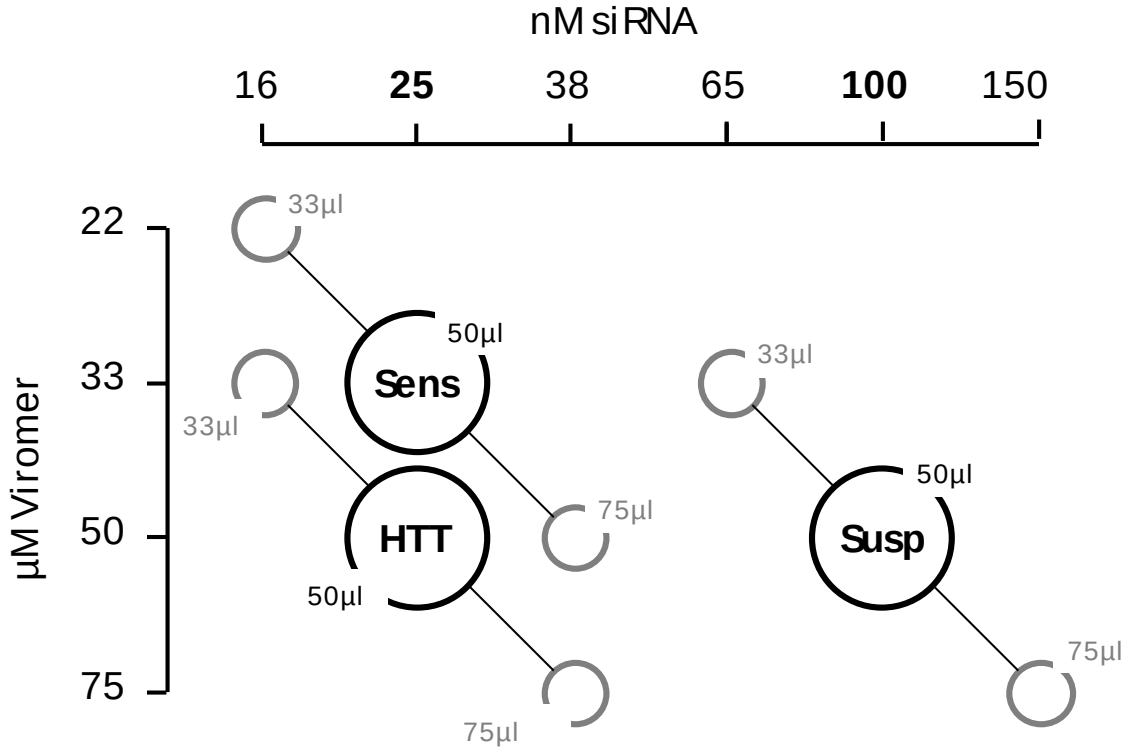
Media: Cells are cultivated in complete medium, which optionally can be changed before transfection. Viromers are fully compatible with cell culture media, sera or antibiotics, so no dilutions or washings are required. Simply keep the Viromers on the cells for the entire experiment.

General Transfection Protocol: Adjust siRNA and Viromer to the concentrations required and combine both for complex formation. Complexation is spontaneous and yields uniform particles.

Warm all reagents to room temperature. The complexation and transfection of Viromers should be done under a sterile workbench using sterile, RNAse free and apyrogenic tips and tubes. Complexes should be prepared freshly.

Finding the Right Conditions

Cells often differ in their response to transfectants, but can be grouped as in the scheme below.



Hard-to-transfect (HTT) adherent cells represent the standard application for Viromers. For HTT's we recommend 25nM siRNA and 50µM of the Viromer (referred to nitrogen) as a starting condition.

Sensitive cells (Sens) such as freshly isolated macrophages or hepatocytes are more sensitive and we recommend reducing the amount of Viromers by 1/3. This is also true for the very accessible cells such as HeLa, CHO or HEK293 which otherwise get over-transfected.

Suspension cells (Susp) or very resistant adherent cells such as primary keratinocytes require about 100nM siRNA and 50µM Viromer.

The 33-50-75µl labels denote the volumes of complexed siRNA required per cavity of a 24-well plate.

Transfection Protocol

The table below provides protocols for the transfection of **HTT**, **Sens**itive or **Susp**ension Cells in 24 and 96-well plates.

		Sens	**HTT**	**Susp**
1	Provide 18µl of siRNA working solution in Buffer F	2.7µM	2.7µM	11µM
2	Dilute x µl Viromer BLUE or GREEN in 495µl Buffer F	3.7	5.5	5.5
3	Add 162µl of the Viromer working solution per 18µl of the siRNA working solution and mix swiftly. Incubate for about 10min at room temperature.			
4	Transfect your cells using x µl for low, mid and high transfer volumes			

	24 well	96 well	siRNA on cells [nM]		
low	33	6.5	16	16	65
mid	50	10	25	25	100
high	75	15	38	38	150

5	Place your cells back into the incubator. Knockdown of the target gene can be assayed 24-72h post-transfection.

siRNA Dose Response Curves

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 dose response experiments, always use the amount of Viromer previously optimized and only vary the siRNA concentration.

The following is a sample protocol for HTT cells which gave about 70% target knockdown using 25nM siRNA in the first experiment.

	siRNA in nM	**50**	**25**	**12.5**	**6**	**3**
1	Provide 5.5µl of x µM siRNA working solution in Buffer F. Use target and control siRNA.	5.5	2.7	1.3	0.7	0.35
2	Dilute x µl Viromer BLUE or GREEN in 545µl Buffer F	6				
3	Add 50µl of the Viromer working solution per 5.5µl of the siRNA working solution and mix swiftly. Incubate for 10min at room temperature.					
4	24-well plates: Transfect your cells using 50µl 96-well plates: Transfect with 10µl					
5	Incubate and analyze knockdown.					

Expert and HTS section

Viromer Technology Info: Particle Formation

Viromers - as most other transfectants - immediately form particles when diluted with buffer. Particle formation is reproducible and defined suspensions are formed even with manual pipetting (fig 1).
The Viromer concentration in buffer however must not exceed 1mM; a threshold above which particles tend to aggregate.

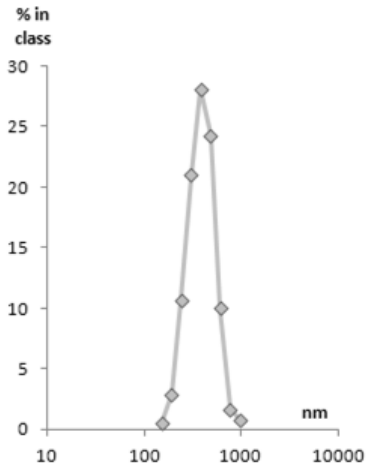


Figure 1: *Particle formation of Viromer BLUE in buffer F. Viromer BLUE at a concentration of 0,56mM forms particles of about 400nm.*

Unlike other transfectants, Viromers remain stable and keep their particle size for hours. This ensures maximum reproducibility within and between experiments (fig. 2).

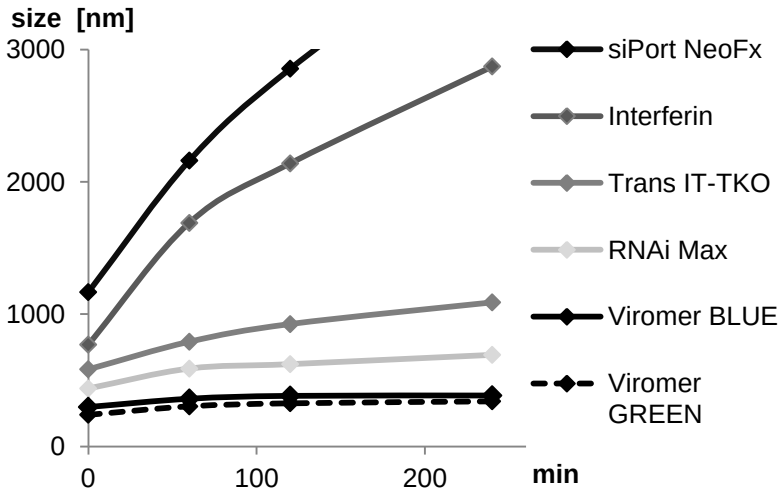


Figure 2: *Particle stability for commercial transfectants or Viromers BLUE and GREEN. Transfectants were diluted according to the instructions and particle sizes were recorded over 240min.*

Viromer Technology Info: siRNA loading and N/P

Viromers bind siRNA through electrostatic interactions between their nitrogens and the phosphate groups on the RNA backbone. The guiding parameter is known as the N/P (nitrogen to phosphate) ratio and **N/P≥12 provides a stable condition** for the system (fig. 3). The resulting particles are slightly positively charged.

Protocol	**N/P**
Sens	32
HTT	48
Susp	12

Viromer stock solution 56mM nitrogen

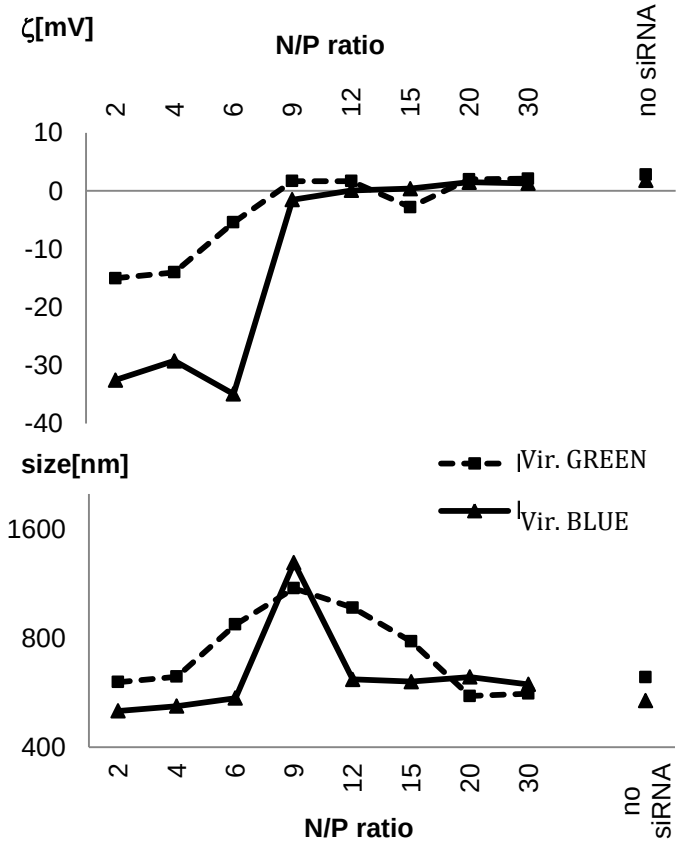


Fig 3: Particle characteristics of siRNA:Viromer complexes

Viromers were prepared according to the manual and loaded with siRNA at various N/P ratios.

Upper panel: *Particles having N/P ≥12 have a low positive charge, those with N/P≤6 are negatively charged.*

Lower panel: *Stable particles are formed with N/P≥12 and N/P≤6, whereas aggregation occurs around N/P=9.*

Charge neutralization between siRNA and Viromers occurs around N/P=9 and results in aggregation. Negatively charged and stable particles are formed at N/P≤6; these are also active in transfection and can be used to minimize the amount of Viromer in the assay.

Doing a thorough optimization

The best way to optimize a transfection experiment is a matrix experiment where Viromer and siRNA are both optimized as independent parameters.
Once the general transfectability of a new cell type has been established under the Sens, HTT or Susp protocol (see Standard Manual) we recommend performing a 3x3 or higher matrix optimization. The following table 1 provides an example of a small matrix for HTT cells centered on the standard condition of 25nM siRNA and 50µM Viromer.
If you optimize under the Susp protocol, please make sure that your N/P values are always greater 12.
In the analysis, calculate Z-scores or p-values to identify the best working area.

N/P ratios		siRNA on cells [nM]		
		12.5	25	50
Viromer on cells [µM]	37.5	72	36	18
	50	96	48	24
	62.5	120	60	30

1	µM siRNA working solution in Buffer F or OptiMEM (3 aliquots of 8µl each)	1.3	2.7	5.5
2	Dilute x µl Viromer with 250µl buffer F		2.1 2.8 3.4	
3	Combine 72µl of each Viromer working solution with the siRNA working solutions and mix swiftly. Incubate for about 10min			
4	Transfect your cells using 50µl per 24 well or 10µl per 96 well			
5	Place your cells back into the incubator			

Table 1: *Protocol for a matrix optimization*

Types of titration protocols

For a first optimization (see: Finding the Right Conditions), a single complexation is carried out and the transfection complex is titrated on the cells by adjusting the transfer volumes. The protocol produces a sharp response curve and gives a good idea of the working point. However, the EC50 values are rather high.

For standard applications or dose response curves, we recommend using a fixed amount of Viromer and titrating the siRNA only. This protocol yields extremely low EC50 values.

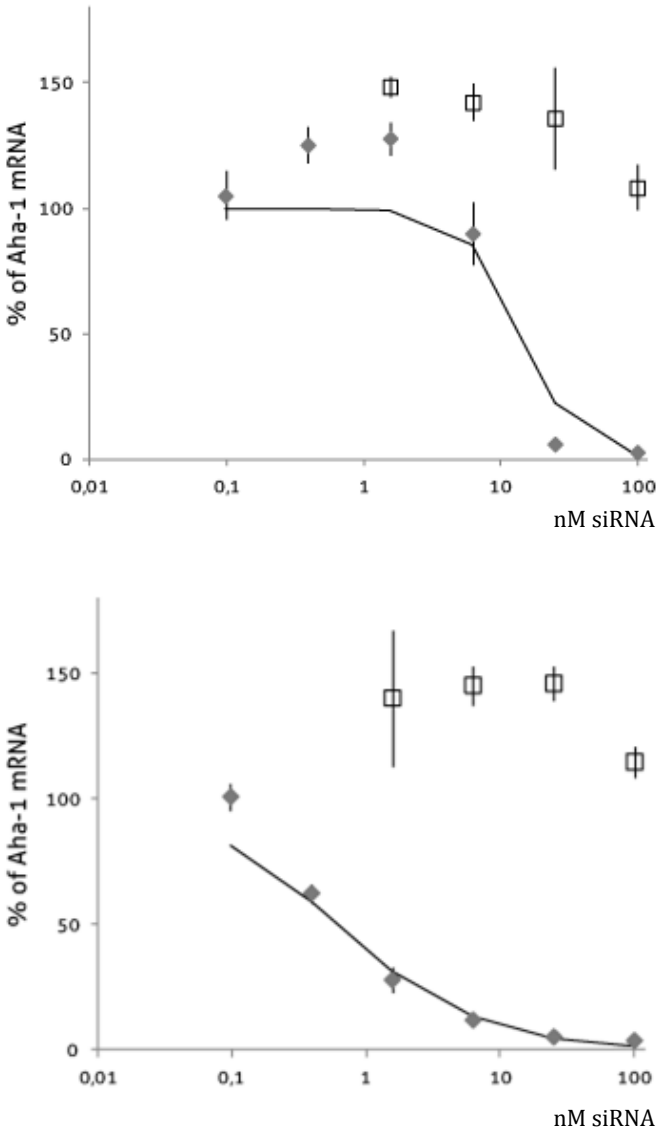


Figure 4:
Protocol comparison in RAW264.7 macrophages.

Both graphs show the dose response of an AHA-1 siRNA. Filled symbols: AHA-1 siRNA, open symbols: Control siRNA

Upper panel: Dose response using different transfer volumes. EC50=14nM

Lower panel: Dose response using a fixed amount of Viromer BLUE. EC50=0.6nM

HTS application development

Viromers are HTS ready due to their full compatibility with sera and culture media and their ability to work in reverse transfection.
Below please find a typical HTS workflow for our standard conditions (HTT protocol using 25nM siRNA with 50µM Viromer).

				96 well	**384 well**
1	Provide plates with siRNA in Buffer F or OptiMEM		siRNA [nM] µl/well	250 10	250 5
2	Dilute Viromer to obtain 500µM working solution.	Use per plate:	µl Viromer µl buffer F	9,80 1090	18,8 2081
3	Dispense Viromer working solution. Pause 10min für complex formation		µl/well	10	5
4	Add cells having a density of		µl/well		
4a	96, adherent	180,000/mL		80	
4b	96, suspension	720,000/mL		80	
4c	384, adherent	90,000/mL			40
4d	384, suspension	360,000/mL			40
5	Place cells back into the incubator				

Troubleshooting

The following steps are recommended for troubleshooting:

1. If transfection was successful but slightly toxic, change the medium 4h after transfection.

2. If there is still toxicity, try a different siRNA. Keep in mind that even non-target control sequences may create background.

3. If there is still no signal, you may want to optimize the time for the analysis of the targeted mRNA or protein. The suggested time point of 24-72 hours is commonly accepted, but the half-life of a specific mRNA or protein may be much longer.

Minimizing Background

Further means to optimize results are:

Cell density: an important parameter to optimize results. Test several seeding densities of the cells depending on growth rate and duration of the experiment. For adherent cells, target about 80% confluency at the time of knockdown analysis.

Type of siRNA: siRNA designs have seen major updates to improve the specificity and reduce immunogenicity. We recommend using siRNA pools and chemically modified siRNA's. If immunogenicity is a concern, monitor levels of central genes, such as OAS1.

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