

VIROMER[®] BLUE

In vitro siRNA/microRNA Standard Transfection

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PRODUCT INFORMATION

General

Technology: Viromer® are polymeric transfection reagents of chemical nature taking advantage of a viral membrane fusion mechanism (hence their name). The “membrane-like” character is provided by alkyl moieties in combination with long chain fatty acids. During endocytosis, Viromer® will become exposed to an acidic environment. The low pH renders the fatty acid moieties uncharged and hydrophobic, a switch that facilitates membrane crossing. This “Active Endosome Escape” technology maximizes transfection efficiency and reduces off-target effects.

Key Benefits:

Active Escape Technology	Efficient for both adherent and suspension cells
Zero Charge	Compatible with serum or antibiotics. Gentle on cells
Stable Particles	Reproducible results
Lipid free	Reliable results, no interference with cell’s lipid metabolism
Reverse Transfection	Ready for High-Throughput Screening

Content: Viromer® BLUE is available in 3 different formats

Viromer® BLUE	50 µl	VB-01LB-00	Incl. Buffer BLUE (10 ml)
	300 µl	VB-01LB-01	Incl. Buffer BLUE (50 ml)
	3 × 300 µl	VB-01LB-03	Incl. Buffer BLUE (3 × 50 ml)

In standard conditions (no optimization), 1 µl of Viromer® BLUE is sufficient for 2 reactions of transfection in the 24-well plate format (incl. both target and control siRNA). The number of transfections that can be performed will depend on the cell type, the optimal transfection scale and the culture plate format (see Tables on Pages 6-7).

Buffer BLUE (pH 7.2 aqueous solution) is required for diluting Viromer® BLUE and the siRNA or microRNA needed to be transfected.

Storage and use: Viromer® BLUE should be stored at **+2-8°C** in the provided aluminum bag. It is then stable for 6 months (#VB-01LB-00) to **1 year** (#VB-01LB-01/#VB-01LB-03). As the reagent is sensitive to atmospheric CO₂, it is recommended to always close the vial and tighten the cap immediately after use. Avoid contact of the reagent with dry ice.

Application: Viromer® BLUE, as Viromer® GREEN, is optimized for the transfection of siRNA and microRNA. For the transfection of plasmid DNA and mRNA, please refer to Viromer® RED and Viromer® YELLOW.

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

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.

BLUE or GREEN?

Viromer® BLUE is a versatile standard with broad support in the user data.

Viromer® GREEN is more selective for particular cells.

Viromer® BLUE and Viromer® GREEN are highly-effective on a wide range of standard and hard-to-transfect cells including **suspension cells**, **stem cells** and **primary cells** without affecting cellular and lipid metabolism.

Please refer to our selection guide at www.viomer-transfection.com

If a cell type is not listed, parallel tests with both Viromer® BLUE and Viromer® GREEN are recommended.

What is different?

Viromer® BLUE and Viromer® GREEN differ in their surface and backbone chemistry but follow the same workflow.

Why testing more than one?

While we have optimized the Active Endosome Escape Technology, Viromer® have no built-in cell specific motifs. Hence their uptake may differ between cell types and we cannot predict in advance which Viromer® would be the best choice.

PROTOCOL GUIDELINES

General Remarks

Conditions of use and required materials:

Warm all reagents to room temperature. The complexation and transfection of Viromer® BLUE should be done under a sterile workbench using sterile, DNase/RNase free and apyrogenic tips and tubes. Complexes should be prepared freshly.

Buffer BLUE (provided in the kit) is required to make dilutions of Viromer® BLUE and siRNA/microRNA.

Media: Viromer® BLUE is fully compatible with all cell culture media, sera or antibiotics, so no dilutions or washings are required. The day before transfection, cells are seeded in complete medium which should be changed before starting (forward transfection).

Forward/reverse transfection: Viromer® BLUE can be used in forward or reverse transfection (page 5). For application in high-throughput screening (HTS), instructions are given at page 9.

Cell Culture and Plating

Grow cells to about **60-80% confluency**. Use the volume of complete medium as mentioned in the table below.

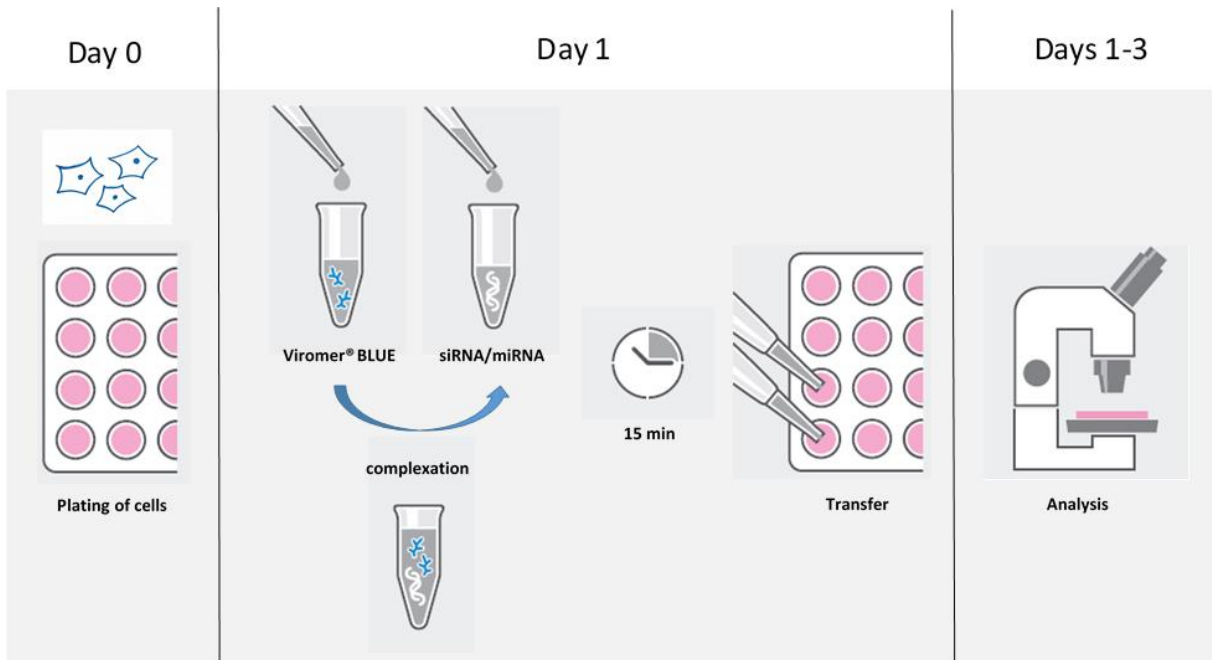
Recommended starting conditions for common cell culture plates are:

Multiwell plate type	96	48	24	12	6
Adherent cells					
Cells seeded per well	12,000	30,000	60,000	125,000	250,000
Range *	±3,000	±10,000	±20,000	±40,000	±80,000
Suspension cells					
Cells seeded per well	48,000	120,000	240,000	500,000	1,000,000
Range *	±12,000	±40,000	±80,000	±160,000	±320,000
Medium per well (ml)	0.1	0.25	0.5	1	2

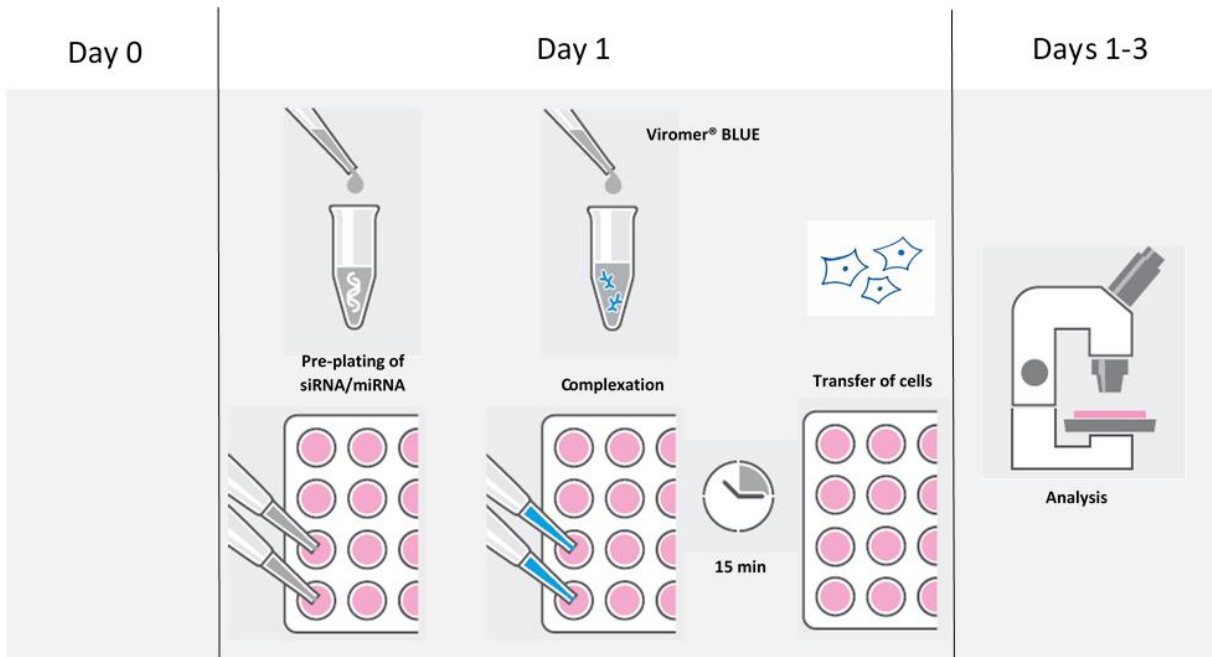
*In reverse transfection protocols, cell numbers should be on the higher end.

Forward/Reverse Transfection: General Workflow

FORWARD TRANSFECTION



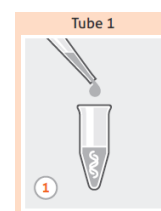
REVERSE TRANSFECTION



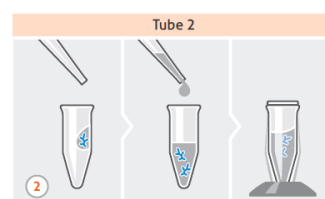
Forward Transfection Protocol: 3-condition optimization

Volumes given here support 24 or 96 well format. For 6 well, scale up 4 fold.

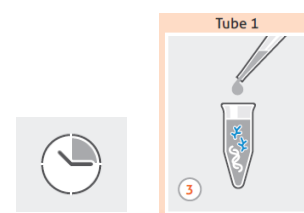
1. Dilute your target and control siRNA/microRNA to **2.8 μM** using Buffer BLUE. For suspension cells, use **11 μM** . Provide a volume of **15 μl** per transfection >> **Tube 1**



2. Place a **3- μl** droplet of Viomer® BLUE onto the wall of a fresh tube. Immediately add **270 μl** of Buffer BLUE and vortex for 3-5 s >> **Tube 2**
Always add Buffer BLUE to Viomer® BLUE, not vice versa!



3. **Complexation:** Pipette **135 μl** of the Viomer® BLUE solution from Tube 2 onto the **15 μl** of siRNA/microRNA solution in Tube 1. Mix swiftly and incubate for about 15 min at room temperature. Repeat with control siRNA/microRNA.



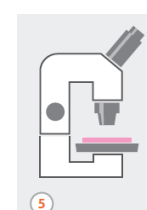
4. Add transfection complexes from step 3 to your cells.



Titrate as per the table below to identify optimal conditions.

Transfection Scale	Volume of complexes to add (μl per well)			siRNA/microRNA on cells [nM]	
	96 well	24 well	6 well	Adherent	Suspension
Low 0.5 \times	5	25	100	13	50
Standard 1.0 \times	10	50	200	25	100
High 1.5 \times	15	75	300	37	150
	$\times 5$ replicates	$\times 1$ replicate	$\times 1$ replicate		

5. Incubate cells under their usual growth conditions. Monitor siRNA/microRNA effects 24-72 h after transfection.



Final Forward Transfection Protocol

During optimization, a specific transfer volume and a specific transfection scale were identified. Please proceed with these specific settings and use the same workflow (step 1 to step 5) as for the optimization protocol for the final transfection protocol.

The table below is a protocol using the **1.0 × Transfection Scale (standard)**. All volumes are given for a single-well format.

Please adjust all volumes according to the optimal transfection scale.

		96 well	24 well	6 well	comments
1.	Start with diluting target and control siRNA/microRNA to 2.8 μM in Buffer BLUE (11 μM for suspension cells)				
2.	Viomer® BLUE (μl)	1	1	4	Buffer BLUE onto Viomer® BLUE Vortex 3-5s immediately
	Buffer BLUE (μl)	90	90	360	
3.	siRNA/microRNA 2.8 μM solution from step1 (μl)	5	5	20	Mix swiftly and incubate for 15 min
	Viomer® BLUE solution from step 2 (μl)	45	45	180	
4.	Transfer volume (μl)	10	50	200	Incubate cells as usual
	replicates	×5	×1	×1	

5. Monitor siRNA/microRNA effects 24-72 h after transfection.

To 100% knock-down... Dose-Response Curves

As a final step in the optimization, the siRNA/microRNA concentration should be limited to the amount necessary to obtain a clear phenotype with maximum separation from any response to a control siRNA/microRNA (e.g. substantial knock-down).

In dose-response experiments, a fixed amount of Viomer® BLUE previously optimized and only vary the siRNA/microRNA concentration.

The following is a standard protocol for adherent cells using 25nM siRNA/miRNA in the initial optimization step (1.0 × Transfection Scale).

	Final siRNA on cells [nM]	50	25	12.5	6	3
1.	Dilute siRNA/microRNA in Buffer BLUE to <input type="text" value="... μM"/> Provide 5 μl of this solution. Use target and control siRNA/miRNA	5.5	2.7	1.3	0.7	0.35
2.	a) Place 3 μl Viomer® BLUE onto the wall of a fresh tube. b) Add immediately 270 μl of Buffer BLUE directly onto the Viomer® BLUE droplet. <i>Always add Buffer BLUE to Viomer® BLUE, not vice versa!</i> c) Vortex 3-5s.					
3.	Add 45 μl of the Viomer® BLUE working solution (from step 2) <u>onto</u> the 5 μl of siRNA/microRNA solution (from step 1). Mix swiftly. Incubate for 15 min at room temperature.					
4.	24-well plates: Transfect the cells using a transfer volume of 50-μl 96-well plates: Transfect the cells using a transfer volume of 10-μl (recommended to do at least 3 replicates)					
5.	Incubate and analyze knock-down of the target gene (24-72h post-transfection)					

High-Throughput Screening (HTS) application development

This section presents a typical HTS workflow for the standard conditions of the Viromer® BLUE protocol (using 25 nM siRNA/microRNA with 50 µM Viromer® BLUE). Based on a common reverse transfection protocol, all steps - complexation, transfer in wells and adding of cells - are performed at the same day following the sequence detailed below.

NOTE:

For **Sensitive** Cells: use 30% less Viromer® BLUE, constant siRNA/microRNA.

For **Suspension** Cells: constant Viromer® BLUE, use 4-times amount of siRNA/microRNA.

			96-well	384-well
1.	Provide plates with siRNA/microRNA diluted in	siRNA [nM]	250	250
	Buffer BLUE	µl/well	10	5
2.	Dilute Viromer® BLUE to obtain 500 µM working solution. Use per plate:	µl Viromer®	9.8	18.8
		µl Buffer BLUE	1090	2081
a) Place <input type="text" value="... µl"/> of Viromer® BLUE onto the wall of a fresh tube.				
b) Add immediately Buffer BLUE onto the Viromer® BLUE droplet.				
Always add Buffer BLUE to Viromer® BLUE, not vice versa!				
c) Vortex for 3-5 s.				
3.	Dispense and mix the Viromer® BLUE working solution and pipette it onto the pre-plated siRNA.	µl/well	10	5
	Incubate 15min for complex formation.			
4.	Add cells having a density of	µl/well		
	96, adherent	250,000/mL	80	
	96, suspension	1,000,000/mL	80	
	384, adherent	125,000/mL		40
	384, suspension	500,000/mL		40

NOTE: For **phenotypic assays** (lasting up to 3 days) plate only 1/3 of the abovementioned cell number. Seed a number of adherent cells sufficient for having a confluency of 90% at the end of the experiment.

5. Incubate cells as usual.

Troubleshooting / Minimizing Background

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/microRNA. Keep in mind that even non-target control sequences may create background.
3. If there is still **no signal**...
→ Increase the incubation time before 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.

Parameters to adjust for minimizing background:

Cell density: 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 knock-down analysis. In case of little to no effects on suspension cells, we recommend to increase the **ratio [concentration of siRNA:Viomer® complex] / [cell density]**; either by reducing the cell density or by increasing the concentration of the siRNA:Viomer® complex.

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.

For additional recommendations, please visit our support pages at www.viomer-transfection.com (incl. updated FAQs) or contact us!

TECHNICAL SUPPORT and ORDERS

Technical Support

Dr. Christian Reinsch
christian.reinsch@lipocalyx.de
+49 345 55 59 620

Info & Customer Service



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

Dr. Olivia Zabel
olivia.zabel@lipocalyx.de
+49 345 55 59 626

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

Mail Orders

order@lipocalyx.de

FAX Orders

+49 345 55 59 846

Webshop

www.viromer-transfection.com

Lipocalyx GmbH

Weinbergweg 23
06120 Halle
Germany