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VIROMER® GREEN

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® GREEN is available in 3 different formats

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

In standard conditions (no optimization), 1 µl of Viromer® GREEN 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 Table on Pages 6-7).

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

Storage and use: Viromer® GREEN should be stored at **+2-8°C** in the provided aluminum bag. It is then stable for 6 months (#VG-01LB-00) to **1 year** (#VG-01LB-01/#VG-01LB-03). As the reagent is sensitive to atmospheric CO_2, 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® GREEN, as Viromer® BLUE, 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® GREEN is tested for transfection using a PLK-1 and control siRNA. Buffer GREEN was analyzed for composition, sterility and RNAse/DNase activity. MSDS are available at www.viromer-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.

GREEN or BLUE?

Viromer® BLUE	is a versatile standard with broad support in the user data.
Viromer® GREEN	is more selective for particular cells.

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

What is different?
Viromer® GREEN and Viromer® BLUE 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® GREEN should be done under a sterile workbench using sterile, DNase/RNase free and apyrogenic tips and tubes. Complexes should be prepared freshly.

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

Media: Viromer® GREEN 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® GREEN 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.

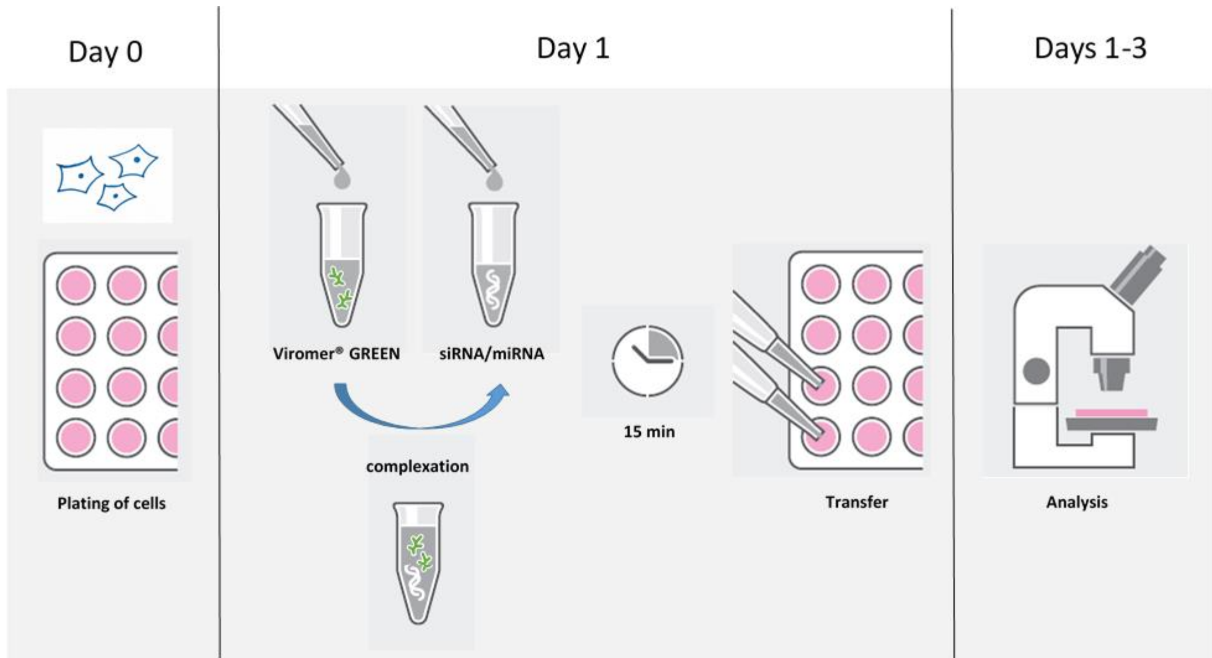
Recommended starting conditions for common cell culture plates are:

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**

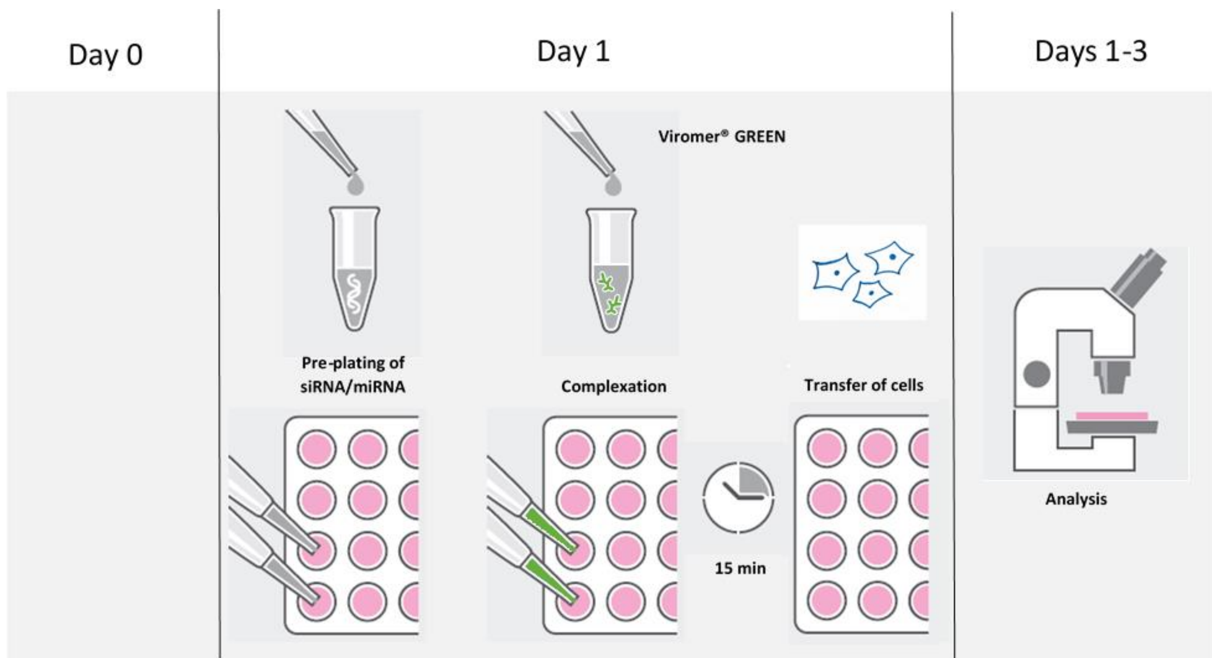
*In reverse transfection protocols, cell numbers should be on the higher end.

Forward/Reverse Transfection: General Workflow

FORWARD TRANSFECTION

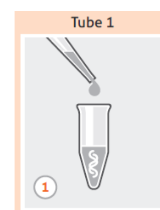


REVERSE TRANSFECTION

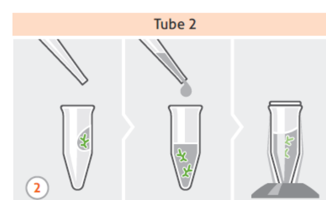


Forward Transfection Protocol: 3-condition optimization

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 GREEN. For suspension cells, use **11 µM**.
   Provide a volume of **15 µl** per transfection >> Tube 1



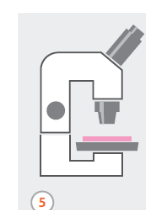
2. Place a **3-µl** droplet of Viromer® GREEN onto the wall of a fresh tube. Immediately add **270 µl** of Buffer GREEN and vortex for 3-5 s >> Tube 2
   Always add Buffer GREEN to Viromer® GREEN, not vice versa!

3. **Complexation**: Pipette **135 µl** of the Viromer® GREEN 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.

		Volume of complexes to add (µl per well)			siRNA/microRNA on cells [nM]	
Transfection Scale		96 well	24 well	6 well	Adherent	Suspension
Low	0.5 ×	5	25	100	13	50
Standard	**1.0 ×**	**10**	**50**	**200**	**25**	**100**
High	1.5 ×	15	75	300	37	150
		×5 replicates	×1 replicate	×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 GREEN (11 µM for suspension cells)				
2.	**Viromer® GREEN** (µl)	1	1	4	Buffer GREEN onto Viromer® GREEN Vortex 3-5s immediately
	Buffer GREEN (µl)	90	90	360	
3.	siRNA/microRNA 2.8 µM solution from step1 (µl)	5	5	20	Mix swiftly and incubate for 15 min
	Viromer® GREEN 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 Viromer® GREEN 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 GREEN to … 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** Viromer® GREEN onto the wall of a fresh tube.
- **b)** Add immediately **270 µl** of Buffer GREEN directly onto the Viromer® GREEN droplet.
  Always add Buffer GREEN to Viromer® GREEN, not vice versa!
- **c)** Vortex 3-5s.

3. Add **45 µl** of the Viromer® GREEN working solution (from step 2) onto 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® GREEN protocol (using 25 nM siRNA/microRNA with 50 µM Viromer® GREEN). 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® GREEN, constant siRNA/microRNA.
For **Suspension** Cells: constant Viromer® GREEN, use 4-times amount of siRNA/microRNA.

			96-well	384-well
1.	Provide plates with siRNA/microRNA diluted in Buffer GREEN	**siRNA [nM]**	250	250
		µl/well	10	5
2.	Dilute Viromer® GREEN to obtain **500 µM** working solution. Use per plate:	**µl Viromer®**	9.8	18.8
		µl Buffer GREEN	1090	2081

a) Place [… µl] of Viromer® GREEN onto the wall of a fresh tube.

b) Add immediately Buffer GREEN onto the Viromer® GREEN droplet.

Always add Buffer GREEN to Viromer® GREEN, not vice versa!

c) Vortex for 3-5 s.

			96-well	384-well
3.	Dispense and mix the Viromer® GREEN working solution and pipette it onto the pre-plated siRNA. Incubate 15min for complex formation.	**µl/well**	10	5
4.	Add cells having a density of	**µl/well**		
	96, adherent — 180,000/mL		80	
	96, suspension — 720,000/mL		80	
	384, adherent — 90,000/mL			40
	384, suspension — 360,000/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:Viromer® complex] / [cell density]**; either by reducing the cell density or by increasing the concentration of the siRNA:Viromer® 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.viromer-transfection.com (incl. updated FAQs) or contact us!

TECHNICAL SUPPORT and ORDERS

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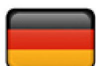

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