

# LabBOOK



## VIROMER<sup>®</sup> CRISPR

for RNP delivery



# General information

## Technology

**Viomer®** are polymer-based transfection reagents featuring a viral mechanism of membrane fusion. They do form transfection complexes with all types of payloads (DNA, RNA, small oligos), which 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 Viomer® surface hydrophobic and facilitates membrane crossing. This "Active Endosome Escape" technology is safe and maximizes transfection efficiency as it uses 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

**Viomer® CRISPR** is optimized for in vitro delivery of Cas9 Ribonucleoprotein (RNP), i.e. pre-complex of the Cas9 endonuclease with one specific guide RNA. Compared to DNA-based approaches, RNP delivery enables faster and higher genome editing, and it offers a powerful alternative for difficult cells.

## Which Viomer® for CRISPR/Cas9 experiments?

If you rather choose to transfect plasmid DNA or mRNA encoding Cas9 protein, we recommend using the **Viomer® RED**. For transfection of guide RNA into Cas9-stable cells, please try **Viomer® BLUE** and **Viomer® GREEN**.

**Success of RNP delivery and subsequent genome editing effect will depend on transfection efficiency. It will vary with cell types and can require optimization.**

**To check Viomer® transfection efficiency by cell type, please consult our cell database online or contact us. For optimization, go further in this manual.**

# Viomer® CRISPR – powerful transfection for genome-editing

Easily accessible genome editing by RNA-guided nucleases has transformed all disciplines of molecular biology, and especially the potential for development of therapeutics is tremendous. Major problems are the fidelity of the system (off-targets), and the efficiency and targeting to specific cell types. Direct delivery of ribonucleoprotein complexes increases fidelity, and also provides a certain degree of control, as it's transient and with no risk of integration into the cell genome.

Whatever the target gene and the final objective of your CRISPR genome editing experiments, you need a reliable and efficient tool for delivering your designed guide RNA and the Cas9 endonuclease into your cells.

While viral transduction, lipofection or electroporation are gold standards in most of CRISPR-Cas9 protocols, there is little consideration for alternative chemical tools. However, polymer-based nanoparticles like the

Viomer® reagents enable high performance transfection with less off-target effects and less impact on cell physiology or viability.

At Lipocalyx, we have listened to researchers looking for other solutions. Our bestseller reagent, the **Viomer® RED**, has proven great efficiency for transfecting CRISPR plasmids or Cas9-mRNA but gave a low output when tested for RNP complex delivery. We therefore screened again over the Viomer® library and worked on formulation to select the best of our technology for that specific purpose. As a result of this optimization effort, **Viomer® CRISPR** is now available to offer the comfort of an easy-to-use and scalable chemical reagent.

**Focus on other key steps, we have the right delivery system for your genome-editing workflow!**



# 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. A specific buffer is provided into the kit for dilution of the **Viomer® CRISPR** reagent and RNP complex.

## Cell Culture and Plating

**Viomer® CRISPR** is compatible with standard cell culture media, serum and antibiotics.

- For forward transfection, seed the cells in complete medium the day before transfection. For reverse transfection, pre-plate your transfection mix and then add the cells (same day).
- 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
<b>Adherent cells</b>			
<b>Cells seeded per well</b>	12,000	60,000	250,000
*Range	± 3,000	± 20,000	± 80,000
<b>Suspension cells</b>			
<b>Cells seeded per well</b>	48,000	240,000	1,000,000
*Range	± 12,000	± 80,000	± 320,000
<b>Medium per well</b>	0.1 ml	0.5 ml	2 ml

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

All recommendations about cell numbers and volumes of **Viomer®** and RNP complex mentioned in the following protocol are given as starting references based on cells used during in-house tests. We highly recommend adjusting RNP preparation and transfection conditions empirically before setting-up any new CRISPR experiment

# Protocol overview: preparation of RNP complex

## Before transfection:

Prepare solutions of Cas9 and gRNA at  $5\mu\text{M}$ . Mix them with an equimolar ratio to form RNP stock solution at  $2.5\mu\text{M}$  (recommended as standard condition).

Use buffer recommended by the supplier(s) to make dilutions. Ideally, testing a range of RNP starting concentrations from  $1.25$  to  $5\mu\text{M}$  will give a broad estimation of achievable genome editing and will help to find the best compromise between rate of delivery, toxic effects and final editing efficiency.

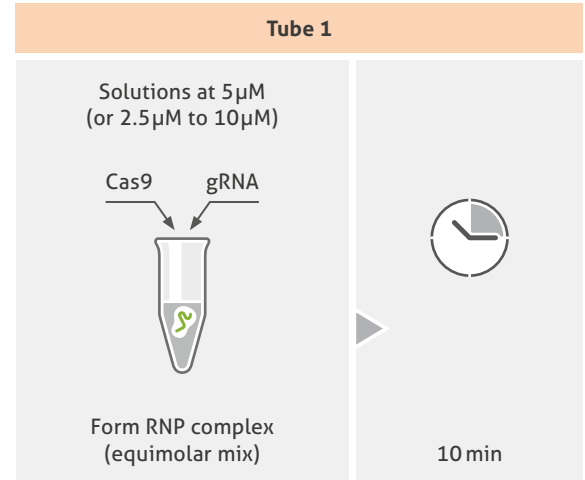
## Tips:

- Design of gRNA is of first importance. It is highly recommended to test at least 3 different sequences for a same target.
- In some cases, RNP complex prepared as non equimolar mix of Cas9 and gRNA can improve efficiency of final genome editing. Excess of gRNA from 1.3:1 to 3:1 can be tested.

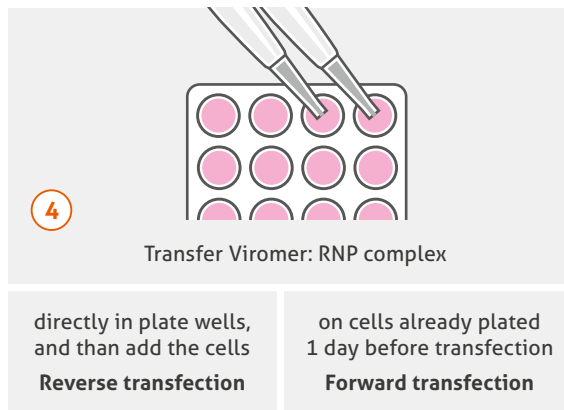
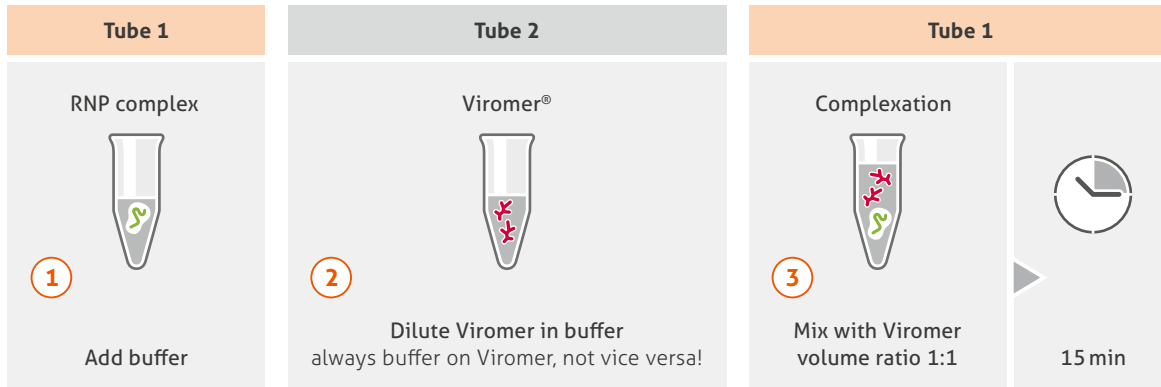
## Reminder

- 1000ng Cas9 eq. to 6.1pmol
- 200ng gRNA eq. to around 6pmol

**To form RNP complex, mix the 2 solutions (equimolar ratio) and wait for 10min at room temperature. You can pursue directly with transfection or store the RNP solution for further use (at  $4^{\circ}\text{C}$  for 2 weeks, or up to 10 weeks at  $-80^{\circ}\text{C}$  without loss in enzyme activity).**



# Protocol overview: Forward and Reverse Transfection



# Volume information for scaling

## To prepare 50µl of Viomer: RNP transfection complex

<b>Tube 1</b>	2.5µl	Cas9 solution (2.5µM to 10µM)*
	2.5µl	gRNA solution (2.5µM to 10µM)*
	20µl	Buffer CRISPR
<b>Tube 2</b>	0.4µl	Viomer® CRISPR
	24.6µl	Buffer CRISPR

\* When starting with 2.5µM solutions, 6.1pmol (1000ng) of Cas9 and around 6pmol (200ng) of gRNA are used. With a ratio 1:1, the formed RNP solution has a concentration of 1.25µM.

## Recommended transfer volumes per well

<b>96-well plates</b>	in 100µl medium	5 to 15µl
<b>24-well plates</b>	in 500µl medium	25 to 75µl
<b>6-well plates</b>	in 200µl medium	100 to 300µl

Use proportional upscaling for larger cell culture formats

# Recommended starting protocol: 3-step titration

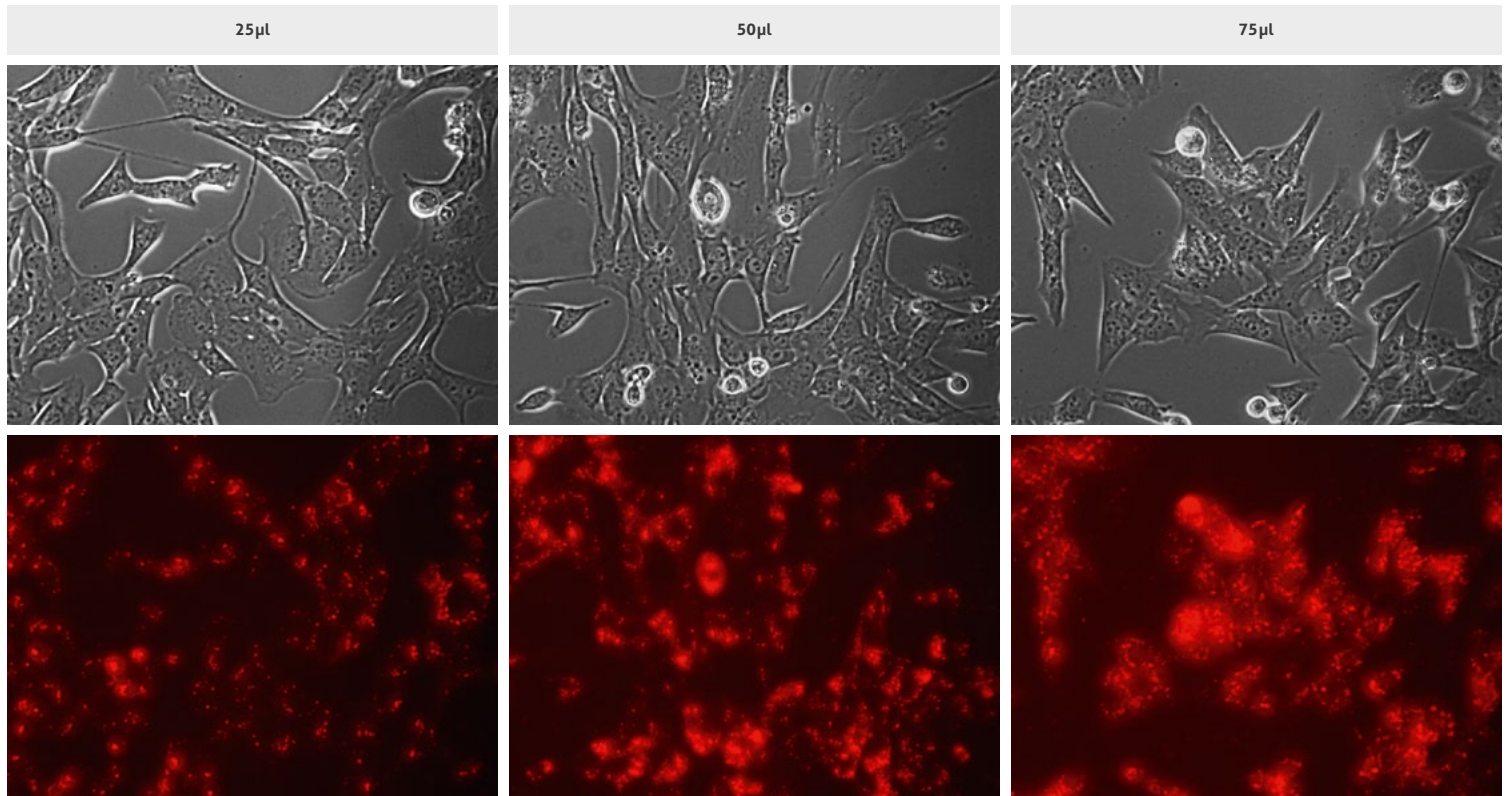
	96-well	24-well	6-well				
<b>1</b>	Mix Cas9 and gRNA into an equimolar RNP complex solution. (1.25µM to 5µM)			<b>Tube 1</b>			
	Cas9	1.5 µl	7.5 µl				30 µl
	gRNA	1.5 µl	7.5 µl				30 µl
	Incubate for 10min at RT and add provided Buffer CRISPR						
	Buffer	12 µl	60 µl	240 µl			
<b>2</b>	Add buffer on Viomer® for dilution (not vice versa!) and vortex 3 - 5 s			<b>Tube 2</b>			
	Viomer®	0.4 µl	1.2 µl				4.8 µl
	Buffer	14.6 µl	73.8 µl				295.2 µl
<b>3</b>	Immediately, combine Viomer® and RNP (volume ratio 1:1). Mix gently by pipetting up and down, shortly spin down the vial. Allow transfection complex formation for 15min.			<b>Tube 1</b>	<b>Tube 2</b>		
<b>4</b>	Tritate the transfection complex on cells using 3 different transfer volumes						
	<b>Transfer volume per well</b>			<b>Final RNP concentration</b>			
	<b>Transfection Scale</b>			stock 1.25 µM	stock 2.5 µM	stock 5 µM	
	0.5 x	5 µl	25 µl	100 µl	6.25 nM	12.5 nM	25 nM
	1.0 x Standard	10 µl	50 µl	200 µl	12.5 nM	25 nM	50 nM
	1.5 x	15 µl	75 µl	300 µl	18.75 nM	37.5 nM	75 nM
<b>5</b>	Monitor genome-editing effects 24 - 72h after transfection						

All volumes are given as exact volumes per well. Please, add 10% extra volume to your mix preparations.

Usable for forward or reverse transfection.

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





Increasing RNP delivery with amount of transfection complex added on cells C2C12/24h post-transfection/labelled gRNA (x20), (24-well format, Alt-R® CRISPR-Cas9 tracrRNA, ATTO™ 550 from IDT, Coralville USA).

Data courtesy of Dr. Laurence Neff, CMU - University of Geneva, Switzerland

**VIROMER<sup>®</sup>**  
**CRISPR**

**[www.viomer-transfection.com](http://www.viomer-transfection.com)**

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