LabBOOK

VIROMER® CRISPR

for RNP delivery

General information

Technology

Viromer® 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 Viromer® 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

Viromer® 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 Viromer® for CRISPR/Cas9 experiments?

If you rather choose to transfect plasmid DNA or mRNA encoding Cas9 protein, we recommend using the **Viromer® RED**. For transfection of guide RNA into Cas9-stable cells, please try **Viromer® BLUE** and **Viromer® 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 Viromer® transfection efficiency by cell type, please consult our cell database online or contact us. For optimization, go further in this manual.

Viromer® 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 Viromer® 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 **Viromer® 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 Viromer® library and worked on formulation to select the best of our technology for that specific purpose. As a result of this optimization effort, **Viromer® 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 **Viromer® CRISPR** reagent and RNP complex.

Cell Culture and Plating

Viromer® 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
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.

All recommendations about cell numbers and volumes of Viromer® 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:

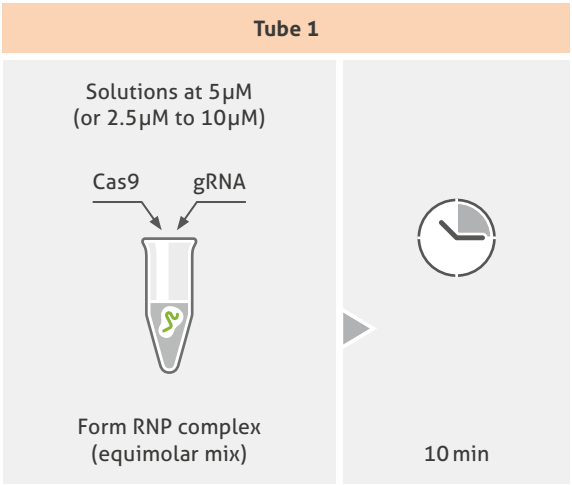
Prepare solutions of Cas9 and gRNA at 5µM. Mix them with an equimolar ratio to form RNP stock solution at 2.5µM (recommended as standard condition).
Use buffer recommended by the supplier(s) to make dilutions. Ideally, testing a range of RNP starting concentrations from 1.25 to 5µM will give a broad 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°C for 2 weeks, or up to 10 weeks at -80°C without loss in enzyme activity).

Protocol overview:
Forward and Reverse Transfection

Tube 1

RNP complex

1

Add buffer

Tube 2

Viromer®

2

Dilute Viromer in buffer
always buffer on Viromer, not vice versa!

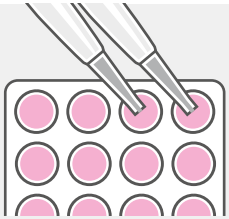
Tube 1

Complexation

3

Mix with Viromer
volume ratio 1:1

15 min



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Transfer Viromer: RNP complex

directly in plate wells, and than add the cells
Reverse transfection

on cells already plated 1 day before transfection
Forward transfection

Analysis

5

Extract DNA and verify genome editing effects 24-72h after transfection

Volume information for scaling

To prepare 50µl of Viromer: RNP transfection complex		
Tube 1	2.5µl 2.5µl 20µl	Cas9 solution (2.5µM to 10µM)* gRNA solution (2.5µM to 10µM)* Buffer CRISPR
Tube 2	0.4µl 24.6µl	Viromer® CRISPR 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		
96-well plates	in 100µl medium	5 to 15µl
24-well plates	in 500µl medium	25 to 75µl
6-well plates	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		
1 Mix Cas9 and gRNA into an equimolar RNP complex solution. (1.25 µM to 5 µM)				Tube 1	
Cas9	1.5 µl	7.5 µl	30 µl		
gRNA	1.5 µl	7.5 µl	30 µl		
Incubate for 10 min at RT and add provided Buffer CRISPR					
Buffer	12 µl	60 µl	240 µl		
2 Add buffer on Viromer® for dilution (not vice versa!) and vortex 3 - 5 s				Tube 2	
Viromer®	0.4 µl	1.2 µl	4.8 µl		
Buffer	14.6 µl	73.8 µl	295.2 µl		
3 Immediately, combine Viromer® and RNP (volume ratio 1:1). Mix gently by pipetting up and down, shortly spin down the vial. Allow transfection complex formation for 15 min.				Tube 1	Tube 2
4 Tritate the transfection complex on cells using 3 different transfer volumes					

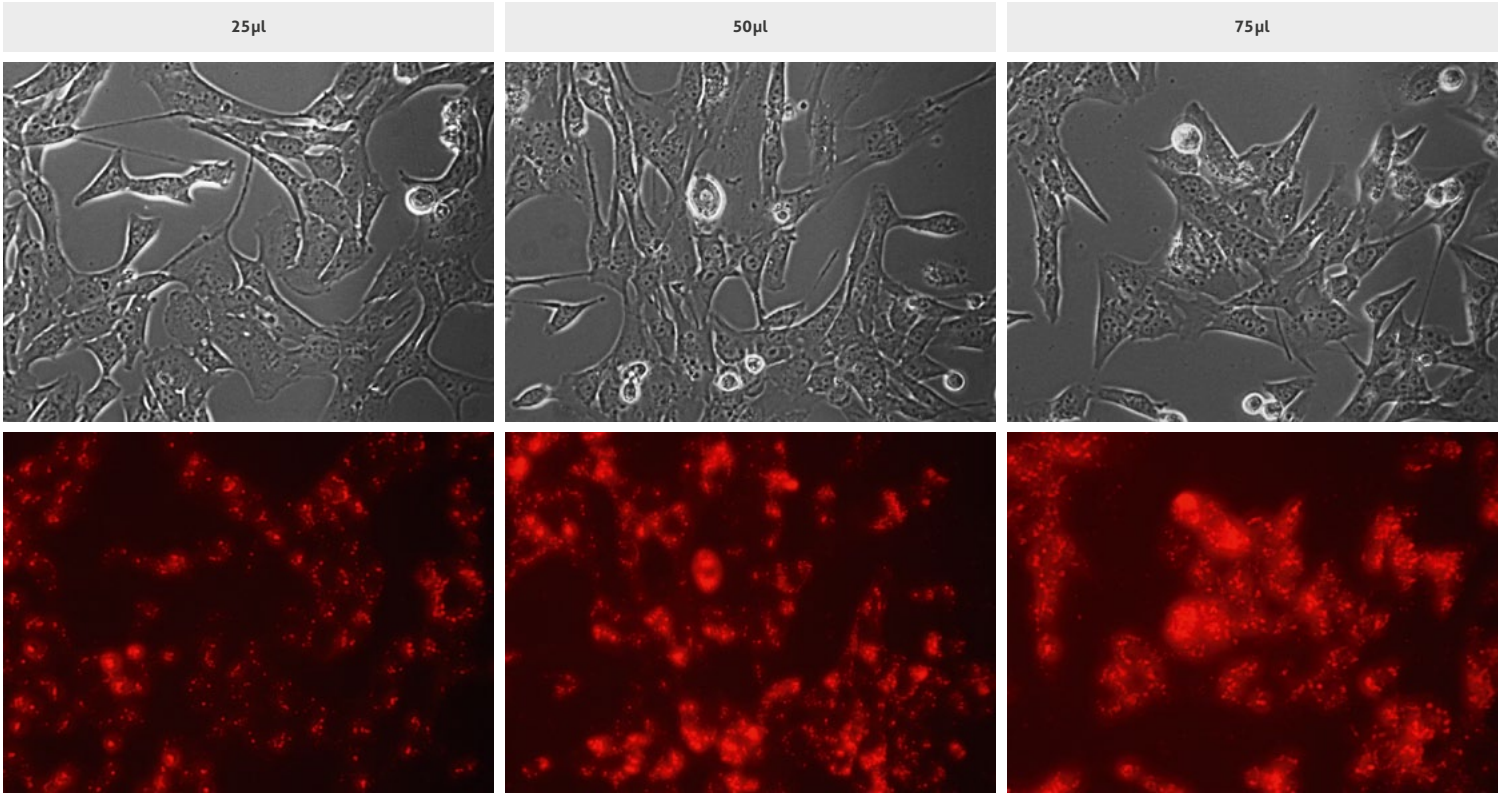
	Transfer volume per well			Final RNP concentration		
Transfection Scale				stock 1.25 µM	stock 2.5 µM	stock 5 µM
0.5 x	5 µl	25 µl	100 µl	6.25 nM	12.5 nM	25 nM
1.0 x Standard	10 µl	50 µl	200 µl	12.5 nM	25 nM	50 nM
1.5 x	15 µl	75 µl	300 µl	18.75 nM	37.5 nM	75 nM

5 Monitor genome-editing effects 24 - 72 h 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®
CRISPR

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2018