Viromer®

RED and YELLOW

in vitro **plasmid DNA** Transfection Protocol

Product Information

This package contains Viromer® RED or Viromer® YELLOW; two new, functionalized transfectants specifically developed for plasmid DNA.

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

Content: Product packages of Viromer® RED or Viromer® YELLOW contain 180µl of liquid bulk transfectant and Buffer E, pH6.0 for dilution and complex formation. Free samples of Viromers® contain 20µl of transfectant and Buffer E.

Reagent use: The product is optimized for the transfection of DNA plasmids or mRNA. For the transfection of siRNA, please refer to Viromer® BLUE, GREEN or BLACK.

Quality control: Each batch of Viromer® RED and Viromer® YELLOW is tested for transfection using a luciferase reporter plasmid. Buffer E was analyzed for composition, sterility and absence of RNAse/DNAse activity. MSDS are available at www.lipocalyx.de.

Storage: Viromers® should be stored between 2-8°C and are stable for 1 year. Please securely 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.

RED or YELLOW?

What is different?

RED and YELLOW differ in their 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.

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 → Data → Plasmid

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:DNA complexes are freshly prepared at the transfection day. Target a cell number that they will be 60…70% confluent at the day of transfection. Recommended starting conditions for common cell culture plates are:

Table1:

Multiwell plate type	**96**	**24**	**6**
Adherent cells seeded per well	12000 ±3000	60.000 ±20.000	250.000 ±80.000
Medium per well (ml)	0,1	0,5	2

In reverse transfection protocols cell numbers should be towards the higher end, **for suspension cells use 4x the numbers** in table 1.

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: In separate tubes, prepare working solutions of DNA and Viromer, respectivly. Combine both for complex formation and add to the cells.

Warm all reagents to room temperature and briefly spin the Viromer tube. Work under a sterile workbench using sterile tips and tubes. Complexes should be prepared freshly.

Standard Transfection Protocol

The following protocol describes the reaction for a 24well format and brings 500ng of DNA to each well.

1. DNA working solution: In a first tube, dilute your plasmid DNA (or mRNA) to 18ng/µl using Buffer E. Provide about 100µl.

2. Viromer working solution: In a second tube, place 5µl Viromer stock; then add 120µl of Buffer E and mix rapidly by pipetting up and down. **Immediately proceed to step 3.** Important: Do not mix the components the other way around as rapid mixing is required for the formation of high-quality particles. Also, do not use any other buffer or diluent for Viromer preparation. Volumes of Viromer stock and Buffer E diluents can be reduced as long as their ratio is kept constant (e.g. 2µl Viromer diluted in 48µl Buffer E).

3. Complexation: Place 15µl of Viromer working solution in a fresh sterile tube, add 85µl of DNA working solution. Again, do not mix the other way around.

4. Mix gently using a pipett and incubate for about 15min at room temperature.

5. Transfection: For cells in a 24-well format, transfer 33µl of the Viromer:DNA complexes per well. This adds 500ng of DNA to your cells.

6. Incubate the cells as usual. Gene expression can typically be monitored after 24…72h.

Complexation (step 3), transfer volumes and resulting DNA amounts (step 5) for different plate formats are listed in table 2.

Table2:	***96-well***		***24-well***		***6-well***	
Step 3						
µl Viromer pre-diluted	3…**4**…5		12…**15**…18		50…**60**…75	
µl DNA/RNA in Buffer E	**22**		**85**		**340**	
Step 5						
µl Transfer Volume / well	6.7	10	33	50	133	200
ng DNA/RNA per well	100	150	500	750	2000	3000

The recommended starting conditions are highlighted, but keep in mind that cells do need optimization for best results. For that, vary the amount of Viromer stock by about ±20% (step 3) while keeping the amount of DNA. In a second step, adapt transfer volumes (step 5).

Direct Complexation Protocol

Another means for optimization is provided by this "Direct Complexation Protocol" as it yields smaller particles than the Standard Protocol.

Direct Complexation Protocol for a 24well format and 500ng of DNA per well:

1. DNA working solution: In a first tube, dilute your plasmid DNA (or mRNA) to 15ng/µl using Buffer E. Provide about 200µl per complexation.

2. Place 1.25µl of Viromer stock in a sterile tube; then add 200µl of the DNA working solution. Important: Do not mix the components the other way around as rapid mixing is required for the formation of high-quality particles. Also, do not use any other buffer or diluent for Viromer preparation.

3. Mix gently using a pipett and allow complexation for about 15min at room temperature.

4. Transfection: In a 24-well format, transfer 33µl of the Viromer:DNA complexes per well. This adds 500ng of DNA to your cells.

5. Incubate the cells as usual. Gene expression can typically be monitored after 24…72h.

Complexation (step 2), transfer volumes and resulting DNA amounts (step 4) for different plate formats are listed in table 3. The recommended starting conditions are highlighted in bold. During optimization, first vary the amount of Viromer stock by ca. ±20% (step 2) and second, adapt the transfer volumes (step 4).

Table3:	***96-well***		***24-well***		***6-well***	
Step 2						
µl Viromer stock	0.5…**0.6**…0.75		1…**1.25**…1.5		2…**2.5**…3	
µl DNA/RNA in Buffer E	**100**		**200**		**400**	
Step 4						
µl Transfer Volume / well	6.7	10	33	50	133	200
ng DNA/RNA per well	100	150	500	750	2000	3000

Troubleshooting

For maximum expression at highest cell viability, we follow these guidelines:

1. When cell viability is low after transfection, reduce the amount of Viromer during complexation as indicated in tab. 2 or 3.
2. When cell viability is high, but target gene expression is low, increase the amount of Viromer during complexation as indicated in tab. 2 or 3.
3. Further, when gene expression is low, increase the plasmid DNA concentration in step 1 of the protocol or, alternatively, increase the transfer volume (in the last step of the protocol) resulting in higher amounts of DNA and Viromer on the cells.
4. If transfection was successful but slightly toxic, change the medium 4h after transfection.
5. Gene expression can be monitored usually after 24h. In the case of low expression you may vary the time point of analysis.

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