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VIROMER® RED

In vitro plasmid DNA and mRNA Standard Transfection

PRODUCT INFORMATION ... 2

GENERAL ... 2

RED OR YELLOW? ... 3

PROTOCOL GUIDELINES ... 4

GENERAL REMARKS ... 4

CELL CULTURE AND PLATING ... 4

FORWARD/REVERSE TRANSFECTION: GENERAL WORKFLOW ... 5

FORWARD TRANSFECTION PROTOCOL: 3-CONDITION OPTIMIZATION ... 6

FINAL FORWARD TRANSFECTION PROTOCOL ... 7

HIGH-THROUGHPUT SCREENING (HTS) APPLICATION DEVELOPMENT ... 8

TROUBLESHOOTING / MINIMIZING BACKGROUND ... 9

TECHNICAL SUPPORT AND ORDERS ... 10

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® RED is available in 3 different formats

Viromer® RED	20 µl	VR-01LB-00	Incl. Buffer RED (10 ml)
	180 µl	VR-01LB-01	Incl. Buffer RED (50 ml)
	3 × 180 µl	VR-01LB-03	Incl. Buffer RED (3 × 50 ml)

In standard conditions (no optimization), 0.6 µl of Viromer® is sufficient for 3 reactions of transfection in the 24-well plate format. 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 Page 7).

Buffer RED (pH 6.0 aqueous solution) is required for diluting Viromer® RED and the plasmid DNA or mRNA needed to be transfected.

Storage and use: Viromer® RED should be stored at **+2-8°C** in the provided aluminum bag. It is then stable for 6 months (#VR-01LB-00) to **1 year** (#VR-01LB-01/#VR-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® RED, as Viromer® YELLOW, is optimized for the transfection of plasmid DNA and mRNA. For the transfection of siRNA and microRNA, please refer to Viromer® BLUE and Viromer® GREEN.

Quality control: Each batch of Viromer® RED is tested for transfection using a luciferase reporter plasmid. Buffer RED 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.

RED or YELLOW?

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

Viromer® RED and Viromer® YELLOW 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® RED and Viromer® YELLOW are recommended.

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

Buffer RED (provided in the kit) is required to make dilutions of Viromer® RED and pDNA/mRNA.

Media: Viromer® RED 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® RED 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.

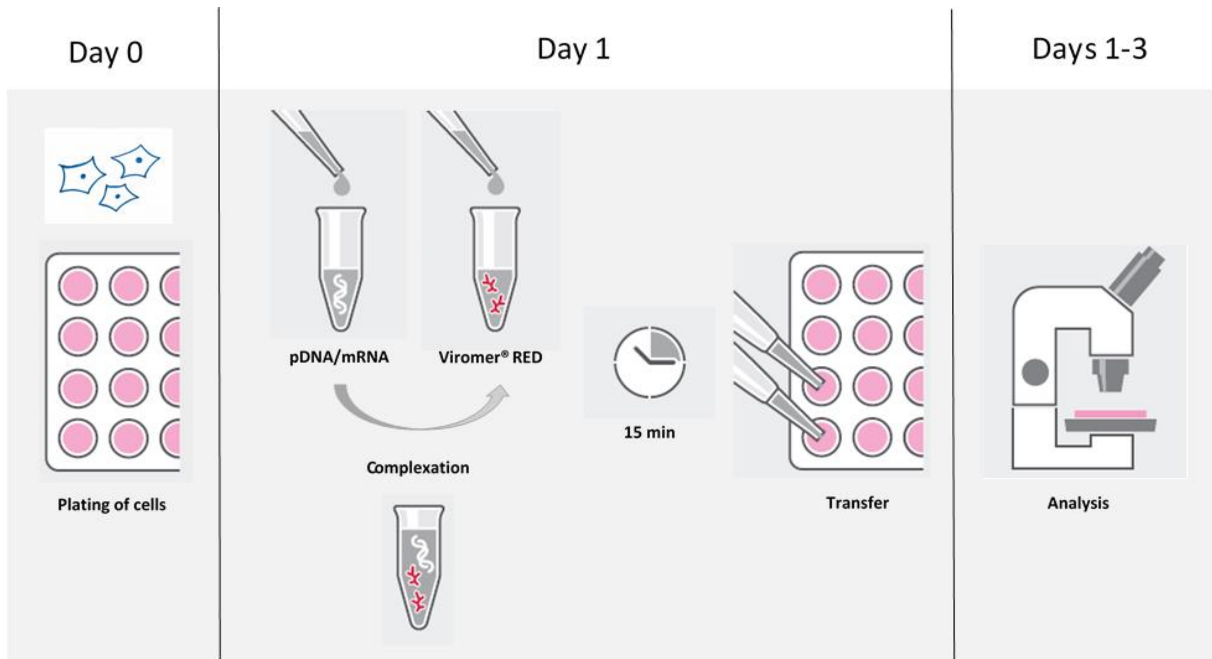
Recommended starting conditions for common cell culture plates are:

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**

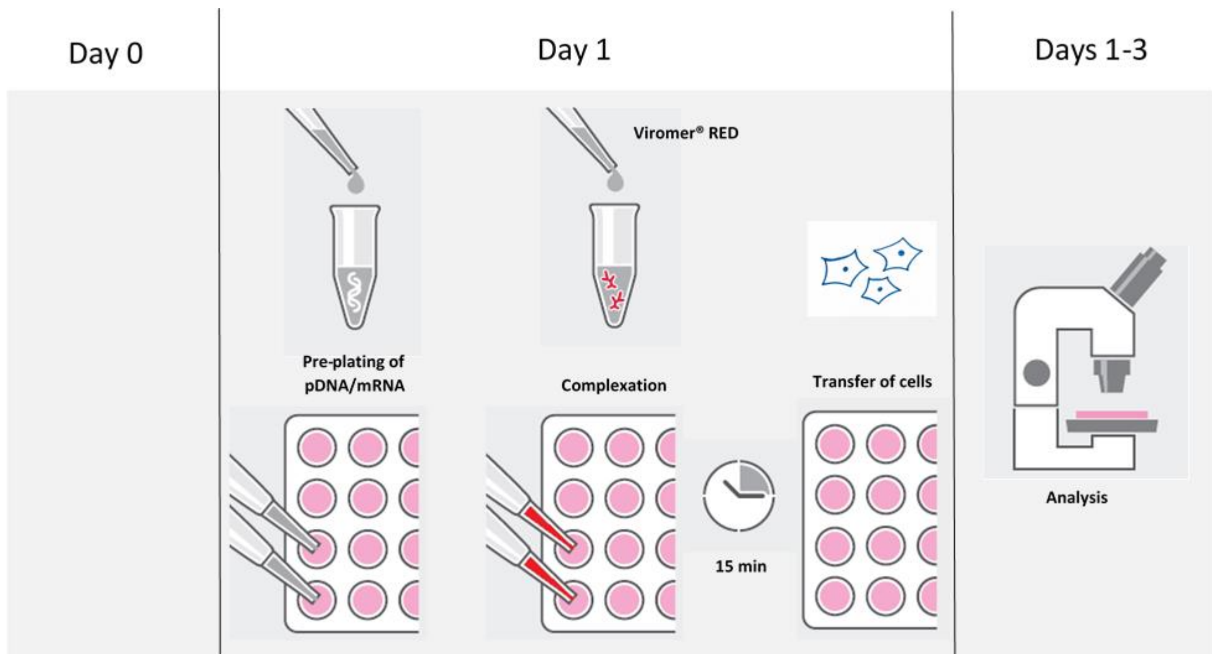
*In reverse transfection protocols, cell numbers should be on the higher end.

Forward/Reverse Transfection: General Workflow

FORWARD TRANSFECTION



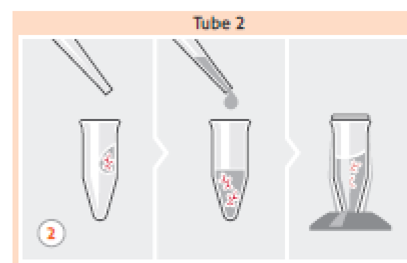
REVERSE TRANSFECTION



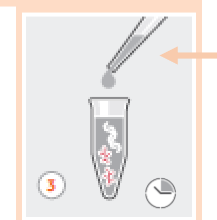
Forward Transfection Protocol: 3-condition optimization

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

1. Dilute your plasmid DNA or mRNA to **11 ng/µl** using Buffer RED. Provide a volume of **135 µl.** >> Tube 1



2. Place a **0.6-µl** droplet of Viromer® RED onto the wall of a fresh tube. Immediately add **14.4 µl** of Buffer RED and vortex for 3-5 s >> Tube 2
Always add Buffer RED to Viromer® RED, not vice versa!

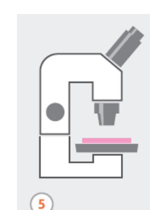


3. **Complexation**: Pipette **135 µl** of the pDNA/mRNA solution from Tube 1 onto the **15 µl** of the Viromer® RED solution in Tube 2. Mix swiftly and incubate for about 15 min at room temperature.

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

Titrate as per the table below to identify optimal conditions.

Transfection Scale		96 well Transfer volume µl	96 well pDNA/mRNA per well ng	24 well Transfer volume µl	24 well pDNA/mRNA per well ng	6 well Transfer volume µl	6 well pDNA/mRNA per well ng
Low	0.5 ×	5	50	25	250	100	1000
Standard	**1.0 ×**	**10**	**100**	**50**	**500**	**200**	**2000**
High	1.5 ×	15	150	75	750	300	3000
		5 × replicates		1 × replicate		1 × replicate	



5. Incubate cells under their usual growth conditions
Monitor pDNA effects 6-24 h after transfection.
Expression from mRNA can begin as early as 2h.

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 pDNA/mRNA to 11 ng/µl using Buffer RED				
2.	**Viromer® RED** (µl)	0.6	0.6	2.4	Buffer RED onto Viromer® RED Vortex 3-5s immediately
	Buffer RED (µl)	14.4	14.4	57.6	
3.	pDNA/mRNA solution from step1 (µl)	135	135	540	Mix swiftly and incubate for 15 min
	Viromer® RED solution from step 2 (µl)	15	15	60	
4.	**Transfer volume (µl)**	10	50	200	Incubate cells as usual
	replicates	15×	3×	3×	

5. Monitor pDNA effects 6-24 h after transfection.
   Expression from mRNA can begin as early as 2h.

High-Throughput Screening (HTS) application development

This section presents a typical HTS workflow for the standard conditions of the Viromer® RED protocol (bringing 100 ng pDNA/mRNA with 1 µl Viromer® RED per well). 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® RED, constant pDNA/mRNA.
For **Suspension** Cells: use 50% more Viromer® RED, constant pDNA/mRNA; increase transfer volume (1,5x)

			96-well	384-well
1.	Provide plates with pDNA/mRNA diluted	**ng/µl**	10	10
	in Buffer RED	**µl/well**	10	5
2.	Dilute Viromer® RED to obtain a working solution	**µl Viromer®**	4.4	8.8
	of **200 µM**. Use per plate:	**µl Buffer RED**	1096	2191

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

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

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

c) Vortex for 3-5 s.

			96-well	384-well
3.	Dispense and mix the Viromer® RED working solution and pipette it onto the pre-plated pDNA/mRNA. Incubate 15min for complex formation.	**µl/well**	10	5
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**…
   → Reduce the amount of Viromer® during complexation.

3. If the cell viability is great, but target **gene expression is low**…
   → Increase the amount of Viromer® during complexation.
   Further, when gene expression is low, increase the pDNA/mRNA 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/mRNA and Viromer® on the cells.

4. If there is still **no signal**…
   → Increase the incubation time before the analysis of the pDNA/mRNA expression. The suggested time point of 24 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, you can target up to **80% confluency** at the time of analysis. In case of little to no effects on suspension cells, we recommend to increase the **ratio [concentration of pDNA:Viromer® complex] / [cell density]**; either by reducing the cell density or by increasing the concentration of the pDNA:Viromer® complex.

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

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