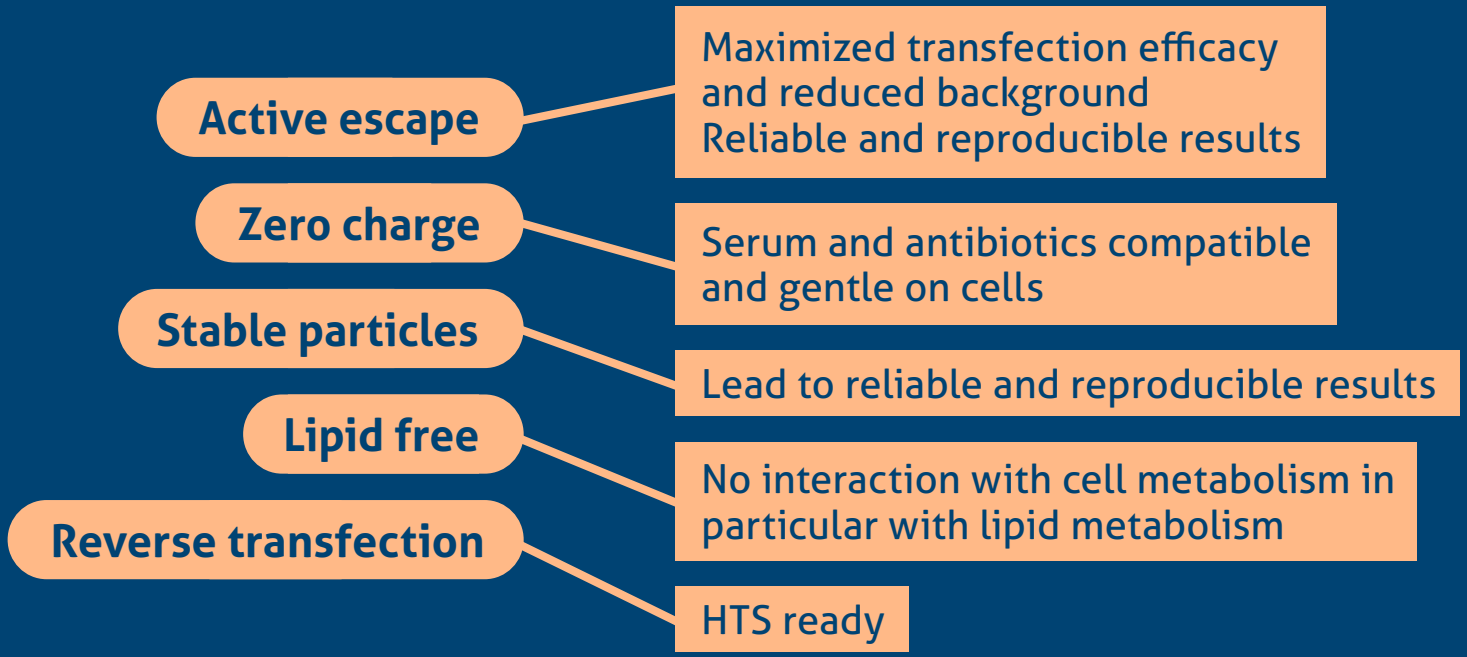
lipocalyx

The Viromer® Factbook

siRNA and plasmid transfection

Fall 2014

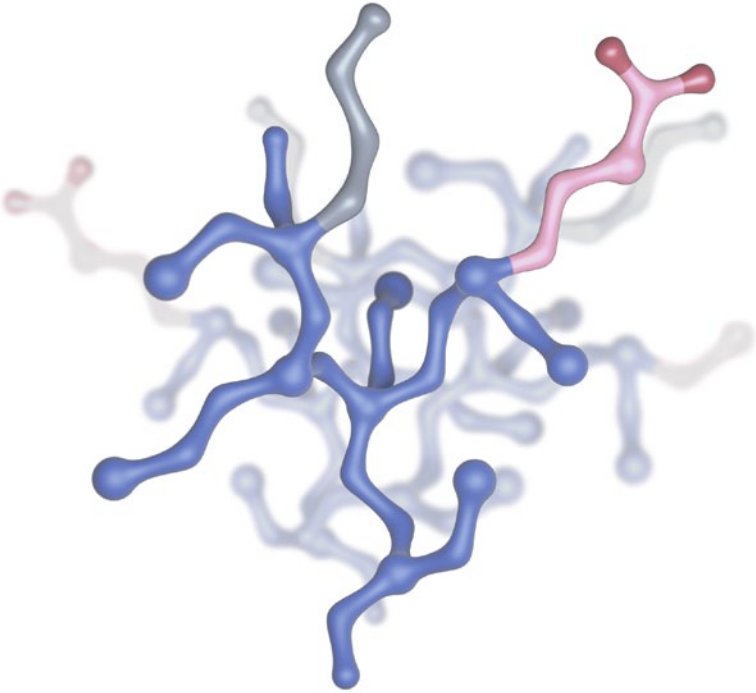
Viromers Features and Benefits



An evolutionary leap for delivering nucleic acids

Transfection should be safe and effective - regardless of cell type or application. However, current methods such as cationic lipofection and aggressive electroporation are damaging to cells, leading to poor viability and inconsistent results.

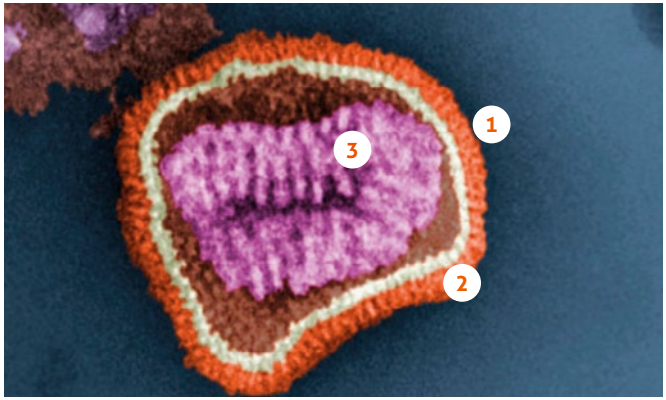
Our novel Viromer technology uses a natural uptake mechanism, designed for a native endosome escape. The new system improves the effectiveness of transfection and allows you to perform functional studies even in challenging cells that were previously difficult to work with... until now.



Viromer Domain Structure. The polymer core (blue) binds DNA or RNA. Hydrophobic and fatty acid groups (grey or red) form a fusogenic coat that becomes active during endocytosis.

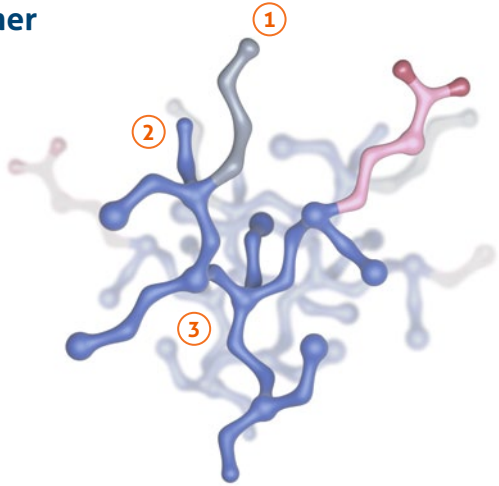
Viromers emulate key features of viral delivery

Virus



Picture: Photo Credit: Cynthia Goldsmith Content Provider(s): CDC/ Dr. Erskine. L. Palmer; Dr. M. L. Martin

Polymer



1 – Endosome escape

The influenza virus hemagglutinin comprises a pH-sensitive fusion domain rich in Ala and Glu residues. The acidic milieu of the endosome triggers membran insertion and an active escape.

2 – Cell binding

The hemagglutinin of influenza is a lectin; it binds to sialic acid residues on the cell surface and triggers internalization.

3 – RNA / DNA binding

In Influenza, the nucleoprotein NP binds RNA in a cationic groove. Multimers of NP are formed through interaction of the tail loop.

1 – Endosome escape

Viromers resemble the fusion peptide with a mix of alkyl (replacing Ala) and pH-sensitive groups (for Glu). Similar to the fusion peptide, these groups trigger an active endosome escape.

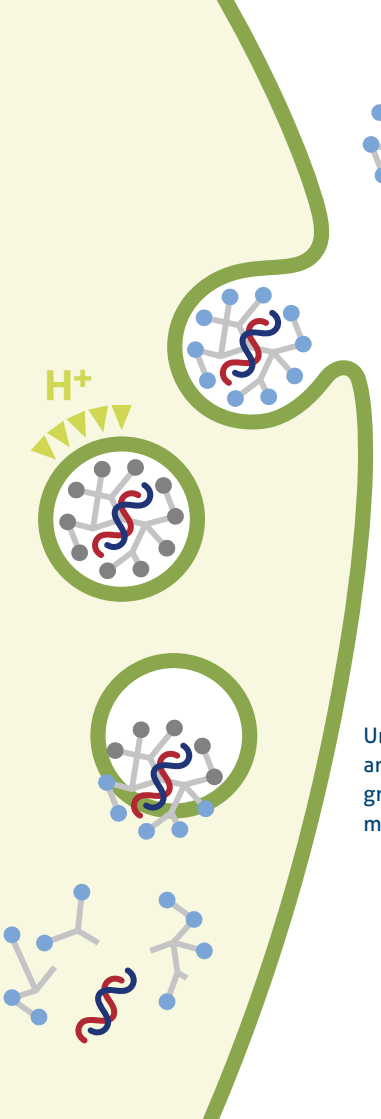
2 – Cell binding

Viromers bind to unknown receptors on the cell surface and enter via the clathrin pathway

3 – RNA / DNA binding

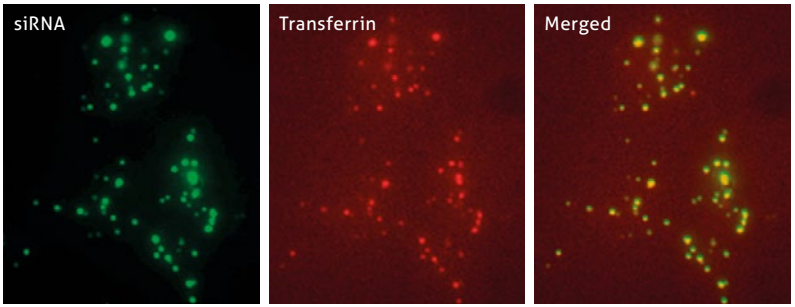
Viromers also bind RNA or DNA through cationic charge groups. Multimerization of Viromers occurs through hydrophobic interaction.

The Viromer uptake pathway



Uncharged (grey) and charged (blue) groups regulate membrane transfer.

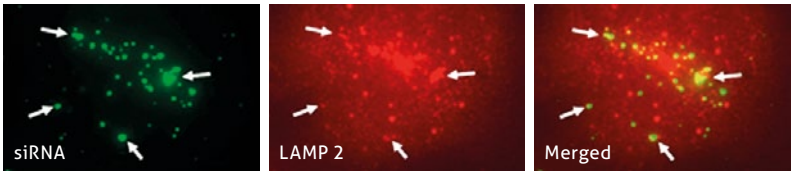
Step 1 The Viromer: siRNA complex is taken up in endosomes



Pictures: Courtesy of Cenix Bioscience

2 hours after transfection, a labelled siRNA does fully co-localize with transferrin, a marker for endosomes.

Step 2 Active Escape. Endosomes acidify which in turn provides membrane-penetrating properties to the Viromers. The Viromer: siRNA complex exits from the lysosomal degradation pathway into the cytosol.



After 6 hours, the siRNA separates well from the lysosomes, here stained with LAMP-2. Active escape has occured and and minimizes the lysosomal degradation.

Step 3 In the cytosol, Viromers regain charge so that no back-transport occurs. The siRNA dissociates from the transfection complex.

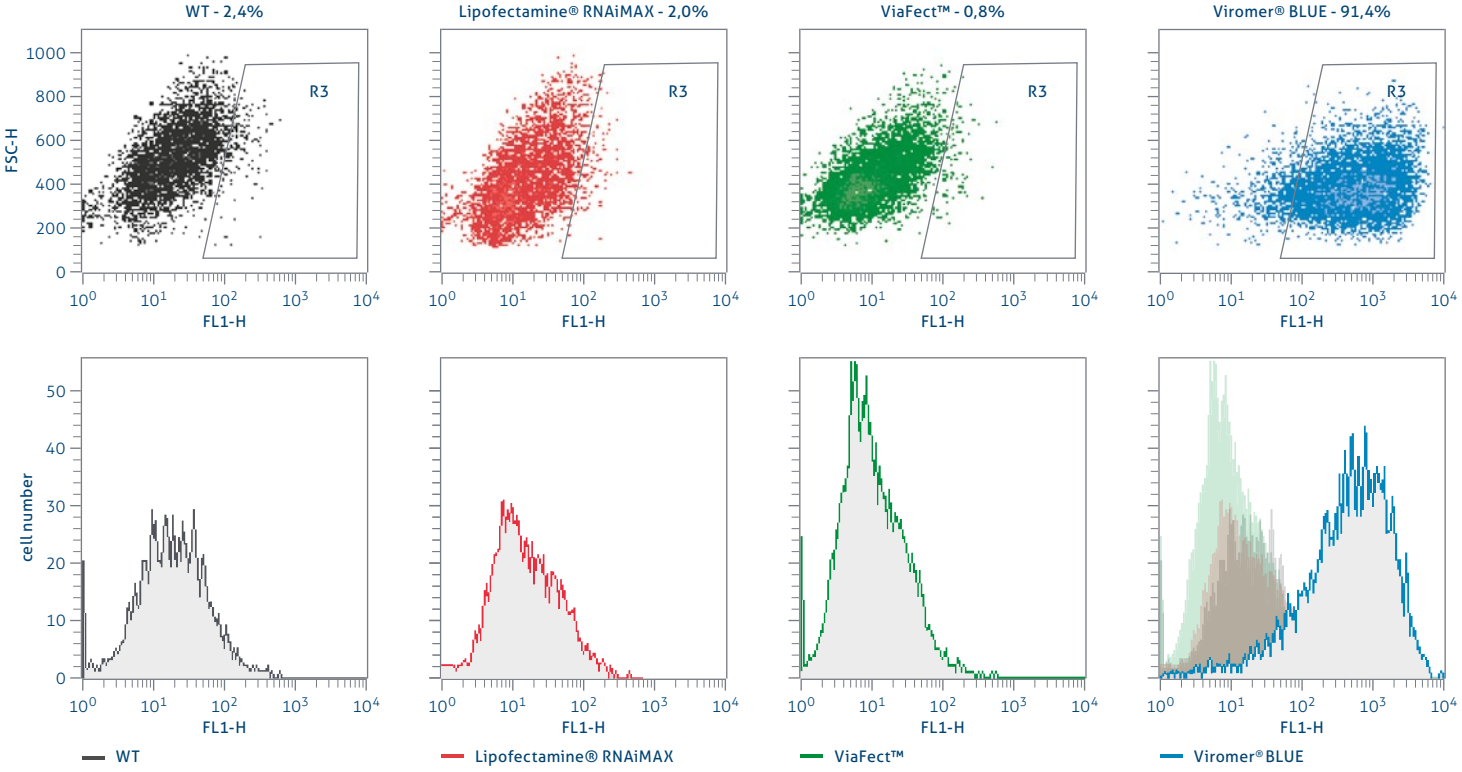
VIROMER® BLUE
VIROMER® GREEN

for siRNA / miRNA transfections

Viromer® BLUE – our most versatile product, non-toxic and highly efficient in standard and challenging cell lines

Viromer® GREEN – a vigorous transfectant with superiority in more specific cells and applications.

Delivery in primary human Mesenchymal Stem Cells



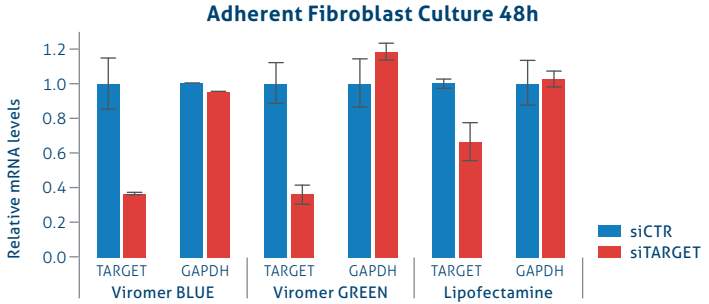
MSC , Fluorescein-labeled Control Oligo

- ViaFect™ and Viromer® BLUE analysed 24h after transfection
- WT and Lipofectamine® RNAiMAX analysed 48h after transfection

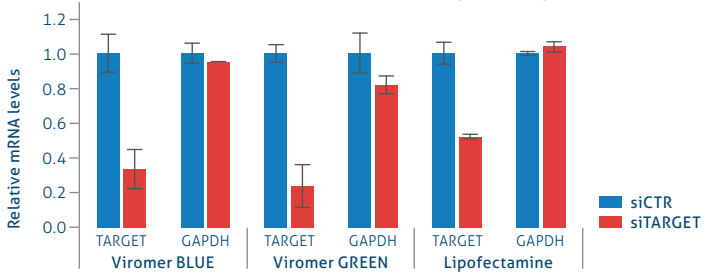
"The Viromer reagent is very encouraging for MSC. By using a FITC labeled control siRNA I got a 92 % transfection efficiency using Viromer Blue. This is an enormous difference compared to promega reagent and lipofectamine."

J. Luetzkendorf, UK Halle (Saale)

siRNA co-culture transfection in primary fibroblasts and Cancer stem cells (3D-mammospheres)



Knockdown of target and control gene in primary mouse fibroblasts transfected with Viromer® BLUE, GREEN or Lipofectamine

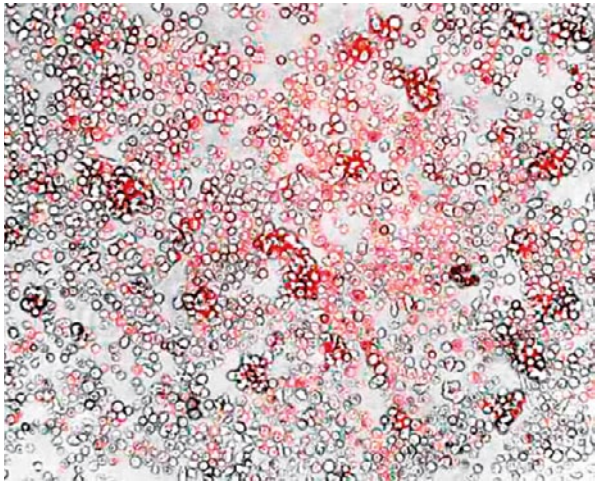


Knockdown of target and control gene in cancer stem cells / mammospheres transfected with Viromer® BLUE, GREEN or Lipofectamine

- co-cultivation in trans-well plates (fibroblasts on top, mammospheres below)
- excellent transfection efficiency with Viromer® BLUE & GREEN compared to Lipofectamine

"And thank you very much for letting us test the Viromer system, we were satisfied with the efficiency of knockdown!"
J. Holland, MDC Berlin

siRNA transfection in disaggregated primary human xenograft tumor cells

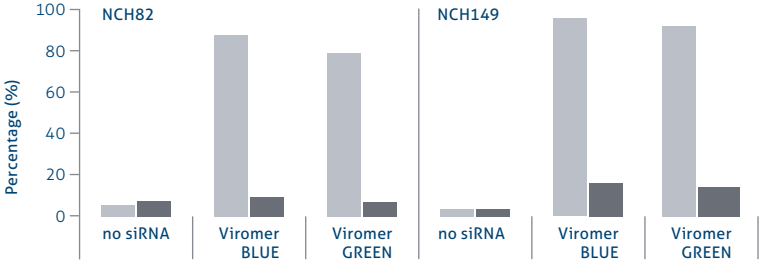


Transfection of siGLO red siRNA in disaggregated primary human xenograft tumor cells with Viromer® BLUE

- disaggregated mass of xenograft cells (stem cells and many others) were transfected with siGLO red siRNA (20nM)
- Viromer® Blue gives almost 100% efficiency

"Note that almost every liposome we tried was either too toxic, killed the very fragile disaggregated tumour cells and were remarkably inefficient at siRNA transfer!"
N. Maitland, University of York

Glioblastoma cells



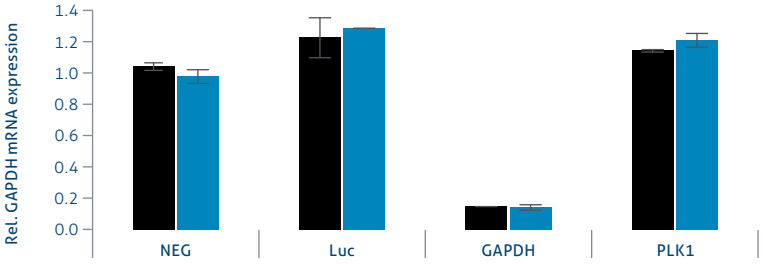
Transfection efficiency (%, grey bars) and Cell death (%, black bars) of glioblastoma cell lines after transfection of siRNAs (at 10nM) using Viromer® BLUE or GREEN.

"The results are more than satisfying, considering that we were not able to transfect these cell lines with any other transfection reagent that is on the market."

I. Dokic, DKFZ Heidelberg

Hs746T- Gastric carcinoma

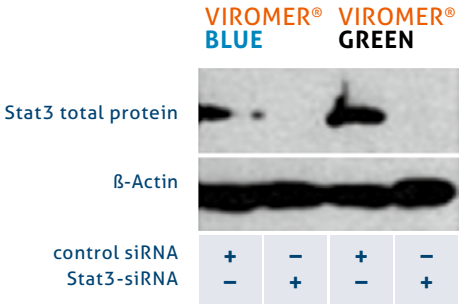
Hs746T cells were transfected with GAPDH siRNA (25nM) for 72h



Both, Viromer® BLUE & GREEN efficiently and safely transfected Hs746T cells. Expression of GAPDH mRNA was reduced by 90% without any signs of toxicity.

CT26 - Colorectal carcinoma

Mouse colorectal carcinoma cells transfected with Stat3 siRNA



Western Blot shows total reduction of Stat3 protein using its siRNA complexed to either Viromer® BLUE or GREEN. Scrambled control siRNA has no effect on Stat3 protein levels.

"We used both Viromers for knocking down Stat3 in CT26 cells and are satisfied with knock-down efficiency. We are delighted from your Viromer Green and would like to test your following products."

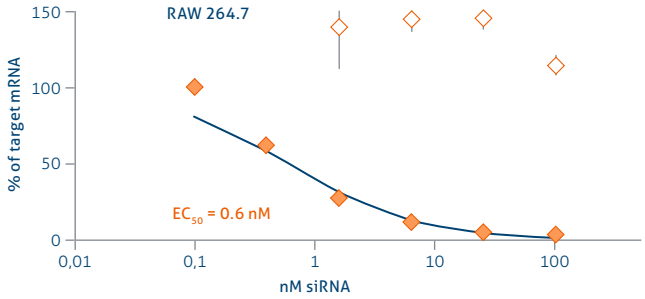
F. Greten, Georg-Speyer-Haus Frankfurt

VIROMERS IN ONCOLOGY

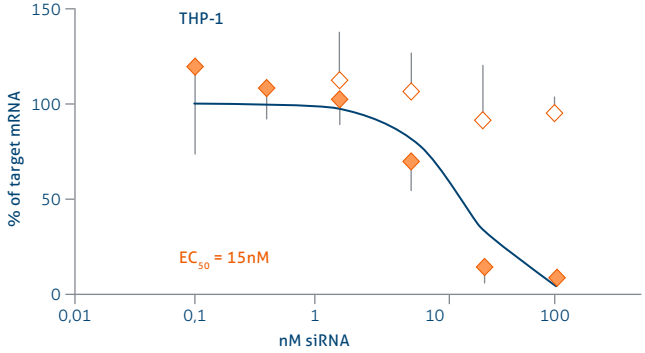
VIROMERS IN IMMUNOLOGY

Delivery into phagocytes

RAW264.7: mouse macrophage-like cell line



THP-1: human monocytic cell line (AML)

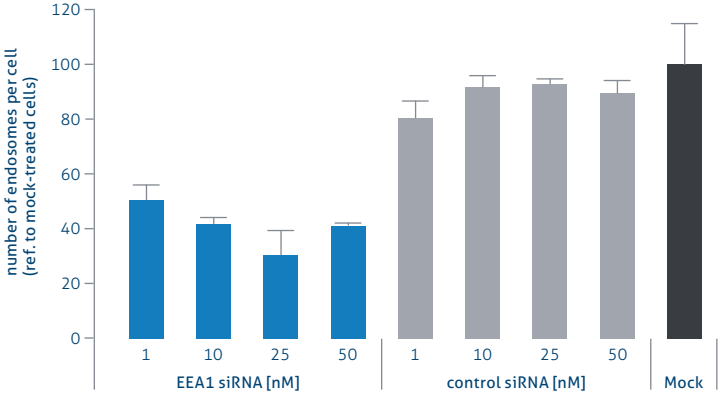


Reduction of AHA-1 mRNA using its siRNA and Viromer® BLUE. Concentrations on the x-Axis in nM, AHA-1 siRNA and control siRNA as filled and open symbols, respectively.

Data collected by Axolabs, Kulmbach

Viromers are HTS ready

Viromers are fully compatible with serum and culture media - no extra washes or media changes. In addition, these novel transfectants show excellent performance in reverse transfection.



Transfection of primary macrophages using Viromer BLUE in a HTS setup

Primary macrophages were freshly isolated from buffy coat PBMC and transfected in a 384-well plate format using siRNA targeting EEA1, which leads to a reduction of endosomes.

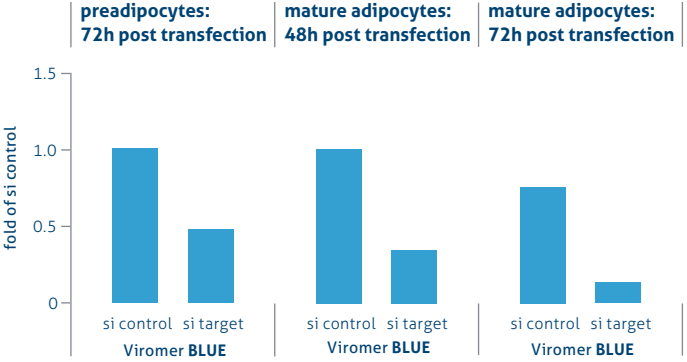
Viromer® BLUE effectively transfects human primary macrophages in a HTS setup.

Reduction of endosomes was followed by image analysis. **Z-scores are -12 and lower.**

„We are absolutely delighted!" M. Bickle, MPI CBG Dresden

Delivery into adipocytes

Transfection of adipocytes or their precursors is a particular challenge. Not only are these cells hard to transfect – being specialized in lipid metabolism adipocytes also react to lipid transfectants. Viromers, due to their polymer nature, cannot interfere with lipid pathways.

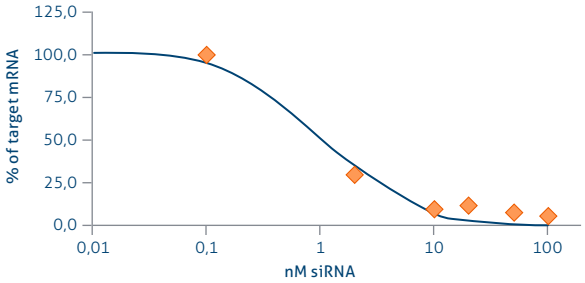


"The results show that for our cells, Viromer Blue is considerably superior to other transfection reagents."
A. Fender, University Hospital Düsseldorf

"Thanks for providing a fantastic reagent. I am looking forward to work with your chemistry."
P. Hallenborg, University of Southern Denmark

Delivery into primary skeletal myoblasts

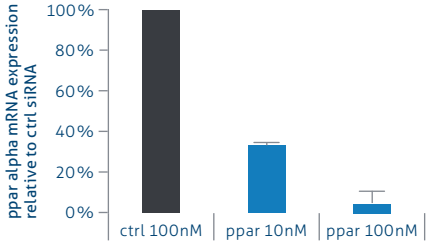
Viromer® BLUE effectively delivers into primary human skeletal myoblasts. Three days after transfection the cells were fully differentiated to myotubes and prepared for RNA analysis.



Data generated by C. Weigert, University of Tübingen

Delivery into primary mouse hepatocytes

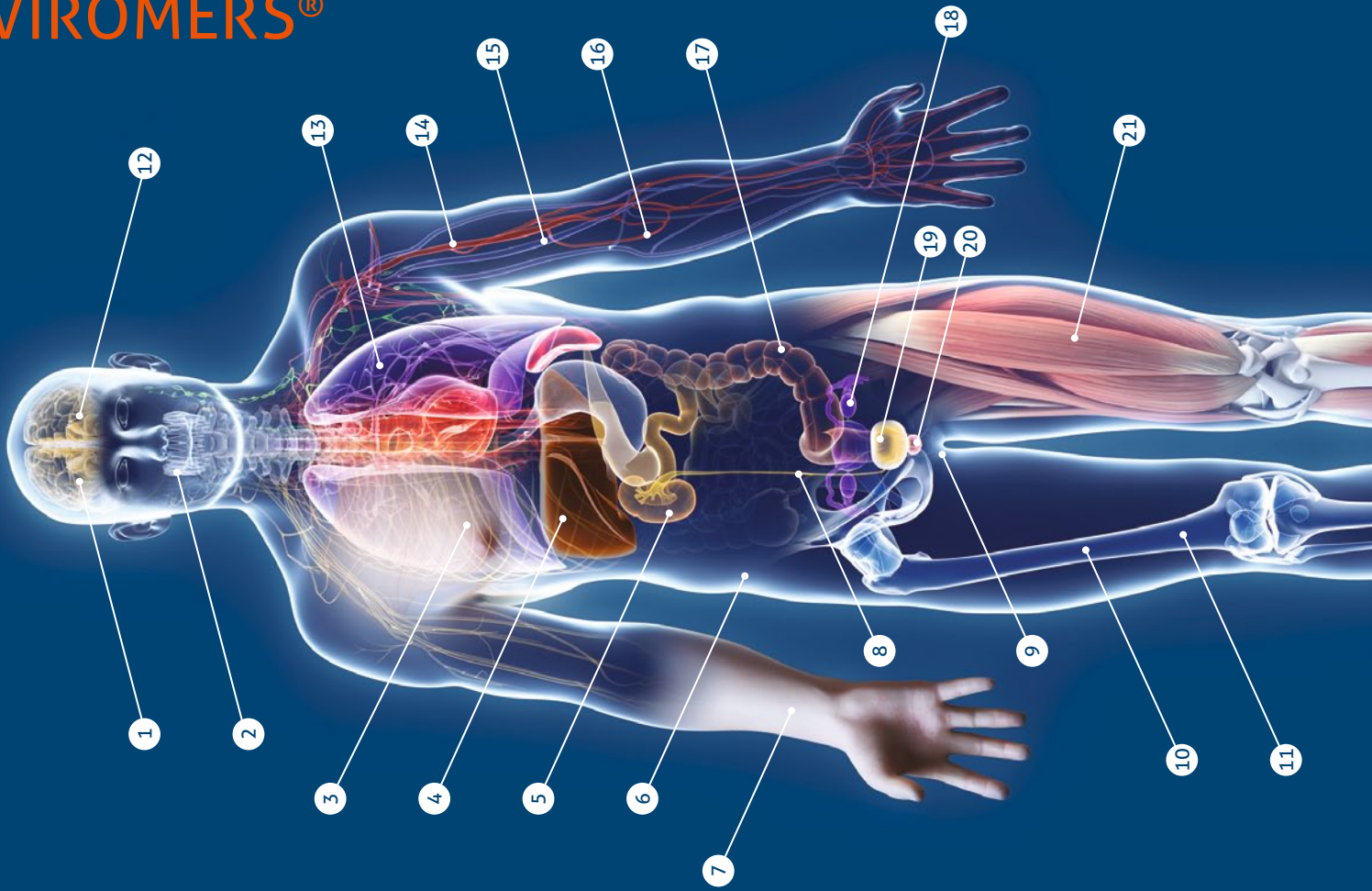
Viromer® BLUE safely delivers siRNAs into primary mouse hepatocytes being highly sensitive for lipofection.



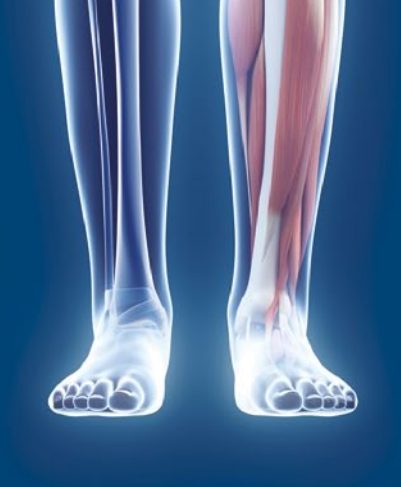
Freshly isolated mouse hepatocytes were transfected with Viromer® BLUE and ppar-alpha (blue bars) or control siRNA (black bar). 24h later prepared for RNA analysis. 100nM yielded a nearly total knockdown.

Data generated by M. Matz-Soja, University of Leipzig.

VIROMERS®



User Atlas of Transfection

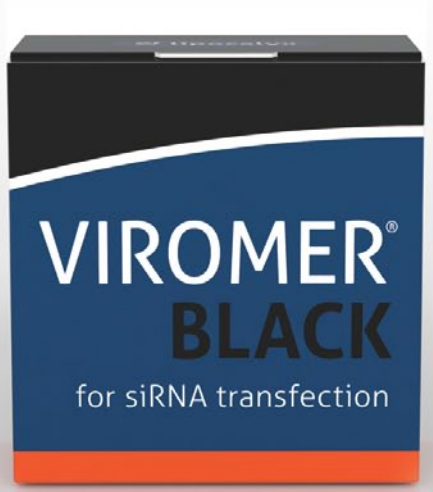


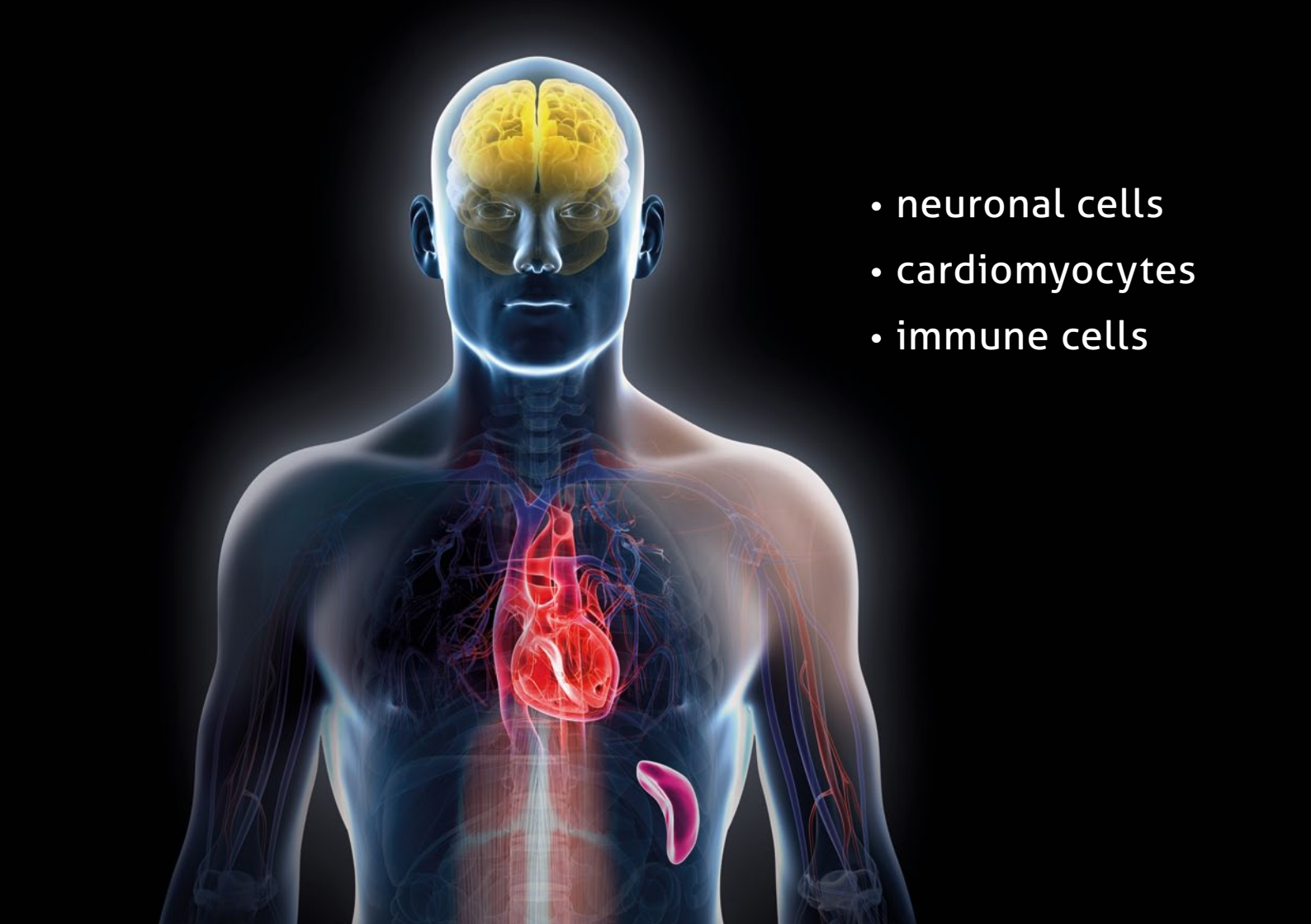
1. Primary neurons C17.2 multipotent neurons Primary Schwann cells
2. Follicle stem cells of wisdom tooth
3. Primary epithelial Mammospheres MDA-MB231 / 468, MCF-7 & SUM159
4. Primary hepatocytes HUH-7 & HepG2 hepatocarcinoma
5. Mouse pheochromocytoma M15 mouse mesonephros HEK-293 & LLC-PK1
6. Mesenchymal stem cells Adipocytes: 3T3-L1 & C3H10T1/2 Mouse embryonal fibroblasts
7. Primary keratinocytes
8. TEU-2 immortalized urothelial cells
9. Ntera
10. U-2 OS bone osteosarcoma
11. Primary bone marrow macrophages
12. Glioblastoma: NCH82 / 149, U87, U-251 MG, LN-229 & A172 Neuroblastoma: SH-SY5Y & Neuro2A
13. MRC-5 lung fibroblasts A549 & H23 lung adenocarcinoma MSTO-211H lung malignant mesothelioma
14. Primary monocytes & macrophages, PBMCs, RAW 264.7 macrophages
15. Leukemia cells: THP-1, MV4-11, Kasumi-4 & JURKAT
16. HUVECs
17. CT26, HT-29, HCT116 &CACO-2 colon carcinoma
18. CHO & CHO-K1 suspension cells
19. Primary bladder smooth muscle cells
20. LNCaP & P4E6 prostate carcinoma
21. C2C12 myoblasts Primary skeletal myoblasts Rat rhabdomyosarcoma cells

VIROMER® BLACK

for siRNA / miRNA transfections

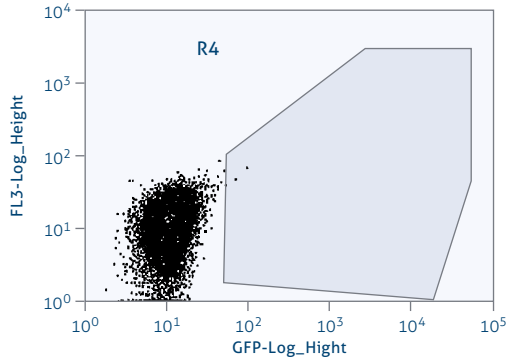
Viromer® BLACK – our latest innovation. BLACK is a premier reagent for the most difficult cells such as cardiomyocytes or neural stem cells.



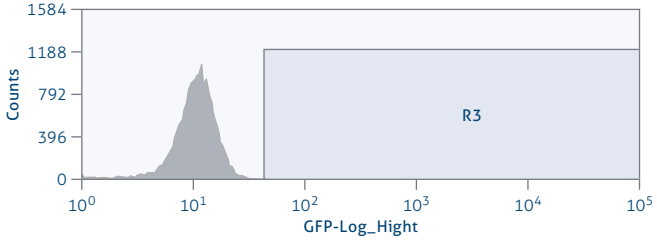

• neuronal cells
• cardiomyocytes
• immune cells

Delivery in adult Neural Stem Cells

Non transfected

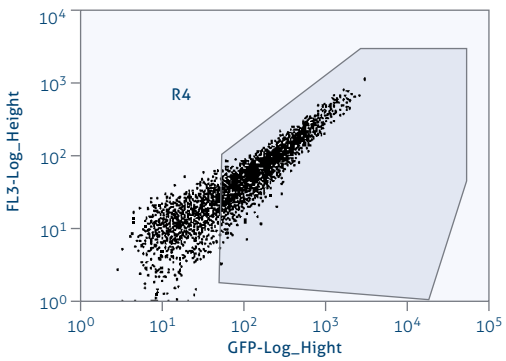


Region	Count	% Hist
Total	16602	100.00
R4	3	0.02

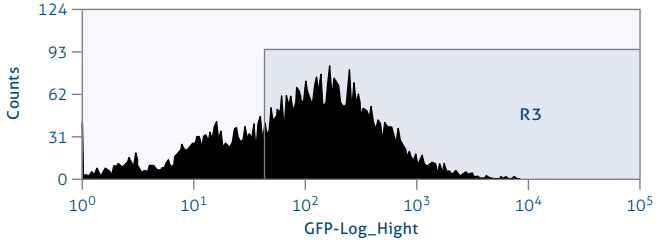


Region	Count	% Hist
Total	19243	100.00
R3	14	0.07

Transfected



Region	Count	% Hist
Total	3829	100.00
R4	2655	69.34



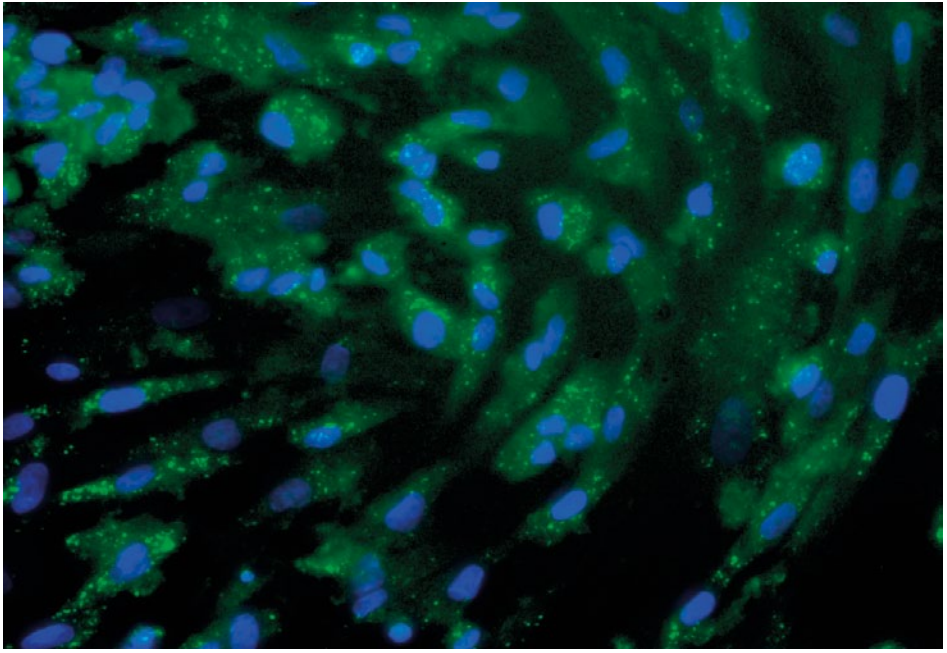
Region	Count	% Hist
Total	3829	100.00
R3	2655	69.34

- adult mouse neural stem cells
- 10-cm dish
- antagomiR 50nM
- Viromer® BLACK 50µM (1:100 dilution)

"...Viromer Black reaches it transfection efficiency at as high as 69.34% compared to our standard transfection method at around 30%."

N. Li, UCL Institute of Neurology London

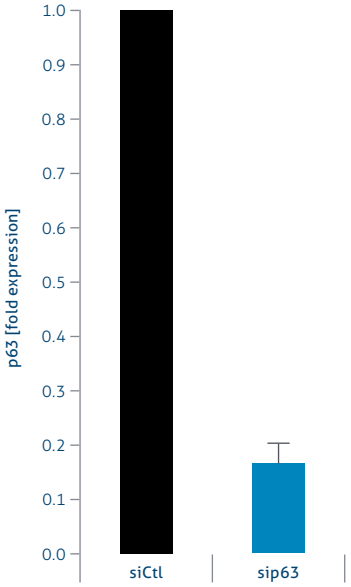
Delivery in human granulosa / luteal cells



- human granulosa / luteal cells
- 96 well setup
- FITC-conjugated signal silence control siRNA (20 nM / 2 x 104 cells / well)
- 100% transfected cells after 48 h

"Viromer Black transfected 100% of the cells after 48 h."
J. Peluso, University of Connecticut Health Center Farmington

Primary keratinocytes



- 48h after transfection
- 5,5pmol sip63
- 84% knockdown

"We are very happy with the results. Now Viromer Black is a very good alternative to our standard transfection method which is associated with the use of high siRNA concentration and significant cell loss. The toxic effect with Viromers is very low and we get a very good cell yield."
University of Regensburg

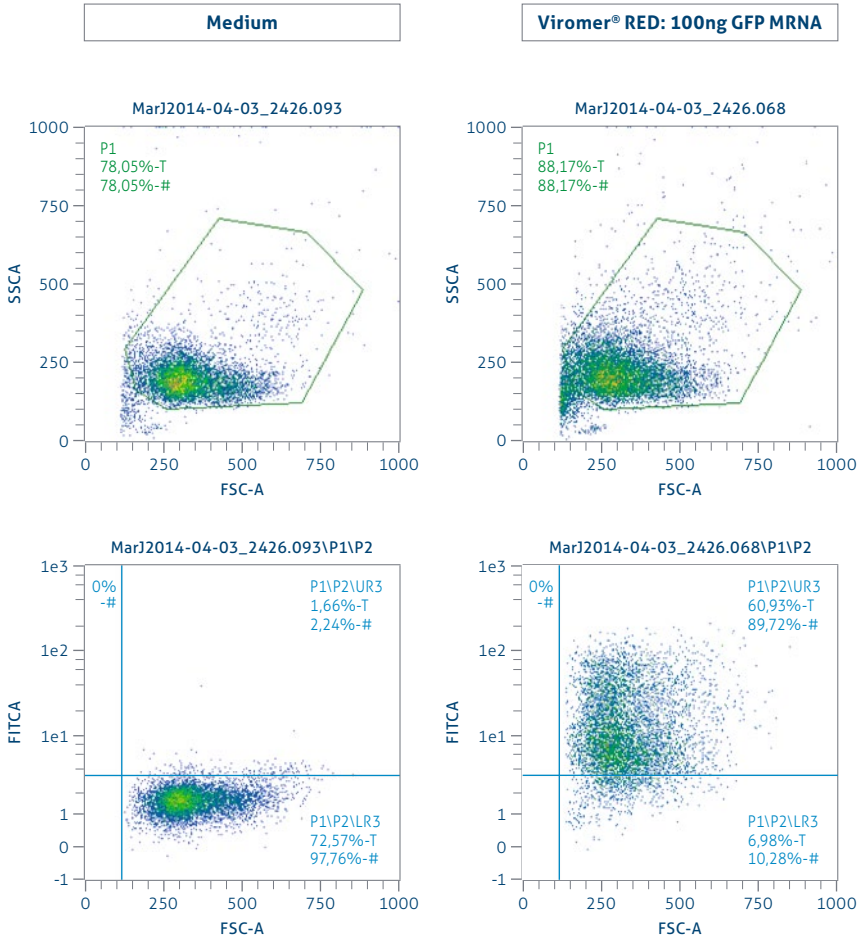
VIROMER® RED
VIROMER® YELLOW

for plasmid DNA / mRNA transfections

In plasmid transfection, we did not witness major innovation for more than a decade. Instead of new reagents, electroporation was introduced for work with challenging cells.

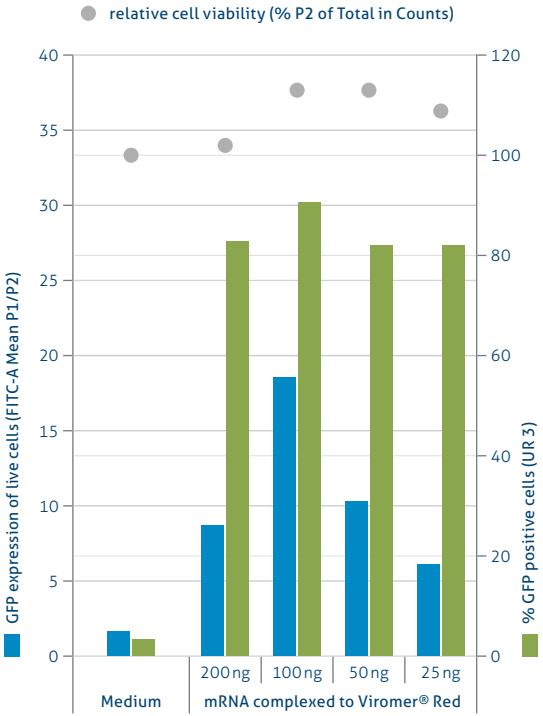
We here present Viromer® RED & Viromer® YELLOW as next generation transfectants for plasmid DNA. As with other Viromers, RED and YELLOW feature a true endosome escape mechanism for the safe and efficient application on your cells.

mRNA transfection in primary monocytes

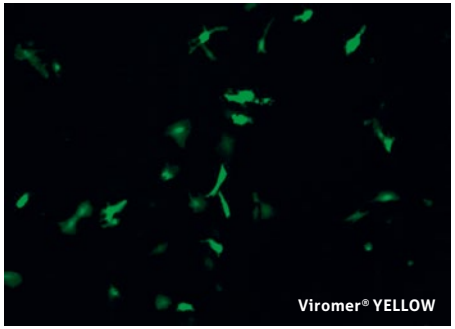


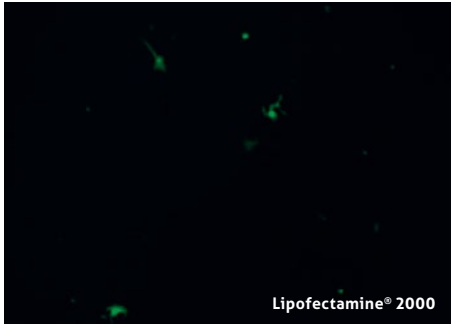
- CD14 purified monocytes from hPBMCs (Buffy Coat)
- 40.000 cells/96-well
- treatment with various doses of GFPmRNA
- GFP detection via MACSQuant

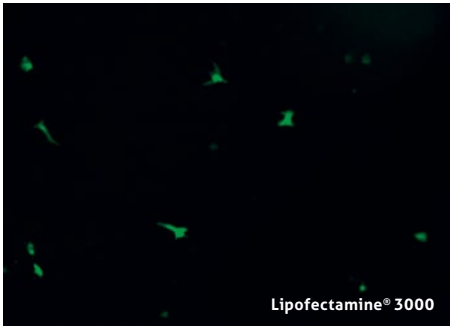
Sum: 90% pos. transfected cells, no visible toxicity



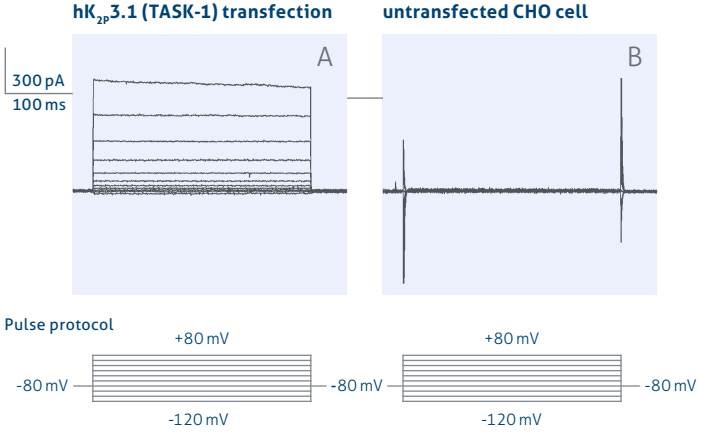
Primary cardiomyocytes





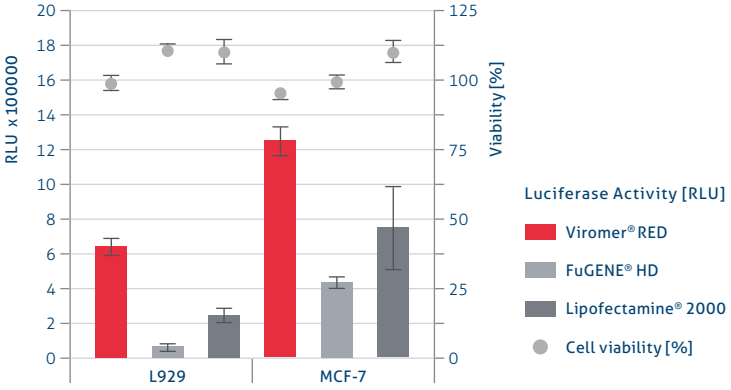


CHO cells



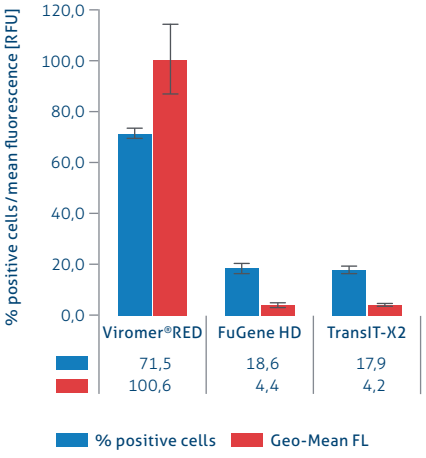
A $hK_{2P}3.1$ (TASK-1) currents in whole-cell patch clamp mode, transfected with Viromer® YELLOW.
B whole-cell patch clamp of an untransfected cell

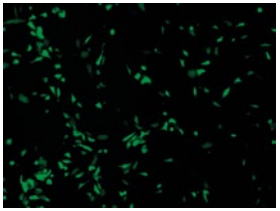
Viromer® RED outperforms major competitors on hard-to-transfect cells.



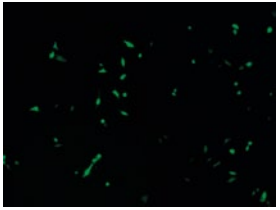
Delivery of luciferase reporter plasmid (65ng DNA/96-well) into L929 mouse fibroblasts and MCF-7 human breast adenocarcinoma cells was highly increased when using Viromer® RED compared to competitors without affecting the cell viability.

SH-SY5Y neuroblastoma



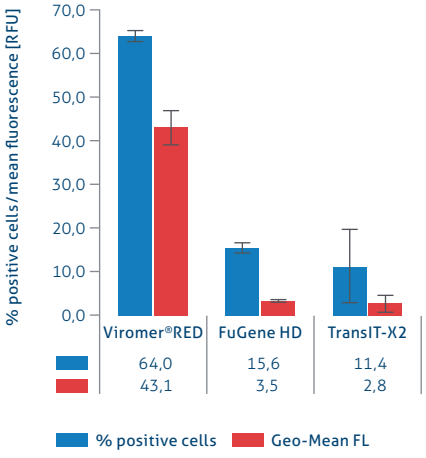


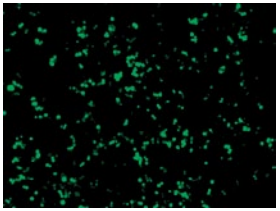
Viromer® RED



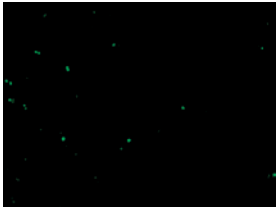
FuGene HD

RAW 264.7 mouse macrophages





Viromer® RED



TransIT-X2

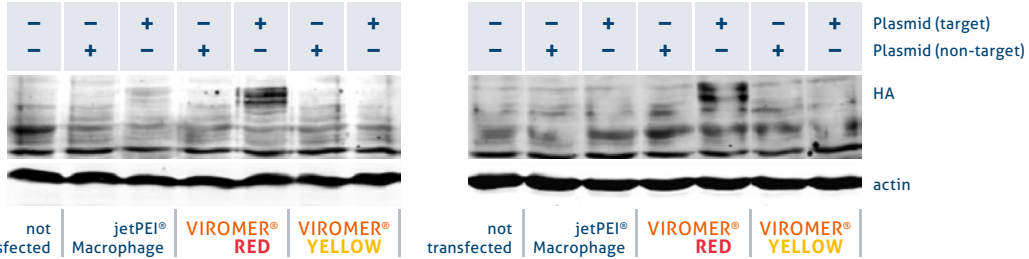
Benchmarking: SH-SY5Y neuroblastoma cells and RAW264.7 mouse macrophages were transfected with eGFP plasmid (100ng DNA/96-well) using Viromer® RED, FuGene ® HD (Roche) or TransIT-X2™ (Mirus) for 24h. FACS and microscopy data demonstrate the superiority of Viromer® RED.

Data generated by H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany

Primary human monocyte derived macrophages

- 6-well setup
- transfected with control plasmid or HA-tagged encoding protein

Only Viromer® RED shows reproducible and satisfactory overexpression of the target protein.



Products & Ordering

Viromers for siRNA/miRNA applications	Quantity	Product Number	Transfections in 24-well
Viromer® BLUE	1 x 0,3ml	VB-01LB-01	500
Viromer® BLUE	3 x 0,3ml	VB-01LB-03	3 x 500
Viromer® GREEN	1 x 0,3ml	VG-01LB-01	500
Viromer® GREEN	3 x 0,3ml	VG-01LB-03	3 x 500
Viromer® BLACK	1 x 0,3ml	VBk-01LB-01	500
Viromer® BLACK	3 x 0,3ml	VBk-01LB-03	3 x 500
Viromers for plasmid/mRNA applications	**Quantity**	**Product Number**	**Transfections in 24-well**
Viromer® RED	1 x 0,18ml	VR-01LB-01	600
Viromer® RED	3 x 0,18ml	VR-01LB-03	3 x 600
Viromer® YELLOW	1 x 0,18ml	VY-01LB-01	600
Viromer® YELLOW	3 x 0,18ml	VY-01LB-03	3 x 600

Technical Support

Dr. Christian Reinsch
+49 345 55 59 620
christian.reinsch@lipocalyx.de

Customer Service and Orders

Bettina Weber
+49 345 55 59 625
bettina.weber@lipocalyx.de

Dr. Olivia Zabel
+49 345 55 59 626
olivia.zabel@lipocalyx.de

Order

order@lipocalyx.de
Fax: +49 345 55 59 846

Webshop

www.lipocalyx.de

lipocalyx

International Distributors

China

Maibio CO., Ltd.
www.maibio.com

麦生物

France

Interchim S.A.
www.interchim.com

interchim

Switzerland

LuBioScience GmbH
www.lubio.ch

lubio science

Taiwan

GenDiscovery Biotechnology, Inc
www.gendiscovery.com.tw

探索生技

United Kingdom & Ireland

Cambridge Bioscience Ltd.
www.bioscience.co.uk

cambridge bioscience

USA and Rest of the World

OriGene Technologies, Inc.
www.origene.com

ORIGENE

Lipocalyx GmbH
Weinbergweg 23
06120 Halle
Germany
www.lipocalyx.de

We are happy to support trials.

order@lipocalyx.de