lipocalyx

THE VIROMER® FACTBOOK

Transfection

of siRNA, mRNA and plasmids

2015/2016

Welcome to Viromer® Transfection Reagents! We provide:

High transfection efficiency

Due to an active escape of Viromer® complexes from the endosome

Great safety

Because Viromer® complexes are non-charged, gentle on cells and compatible with serum and antibiotics

Easy and fast transfection with consistent results

Ascribed to straightforward protocol including initial optimization

All features combined generated excellent results in challenging cells such as primary- and suspension cultures, macrophages and stem cells shown in this booklet. We thank our supporters for sharing their valuable data with us and the growing community of Viromer® users.

- **New to Viromers? Our Start Positive® controls make it easy to begin with, see page 16**
- **Want to learn about the Viromer technology? page 20 has the answers**
- **Product information, support and our list of distributors to be found on pages 24 - 27**

Transfection of siRNA and miRNA

VIROMER® BLUE

Versatile for standard and challenging cells

VIROMER® GREEN

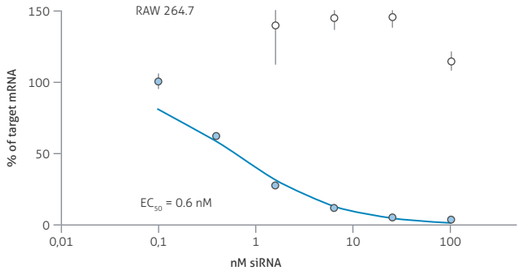
Selected for specific cells such as THP-1, fibroblasts, and colon carcinoma

VIROMER® BLACK

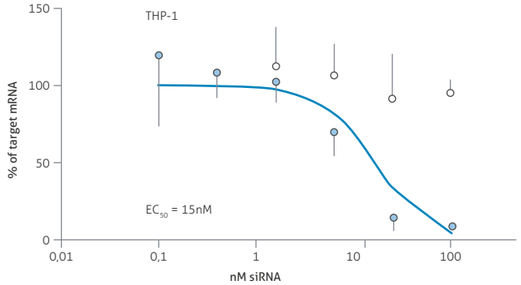
Optimized for very challenging cells such as neural stem cells and primary keratinocytes, unique for transfection of antagomiR

Viromer® BLUE – Very Efficacious Knock-Down in Macrophages and THP-1 Cells

RAW264.7: Mouse macrophage-like cell line



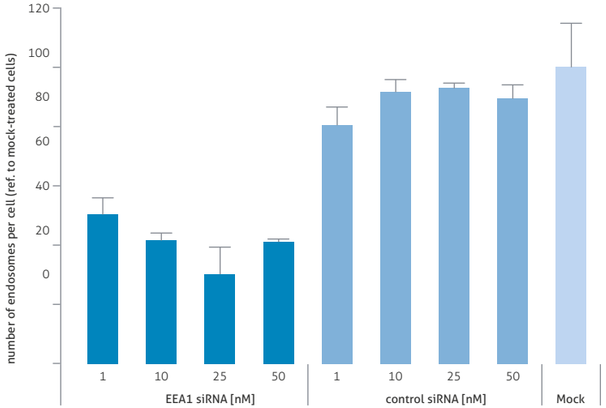
THP-1: Human monocytic cell line (AML)



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, Germany

Human primary macrophages



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

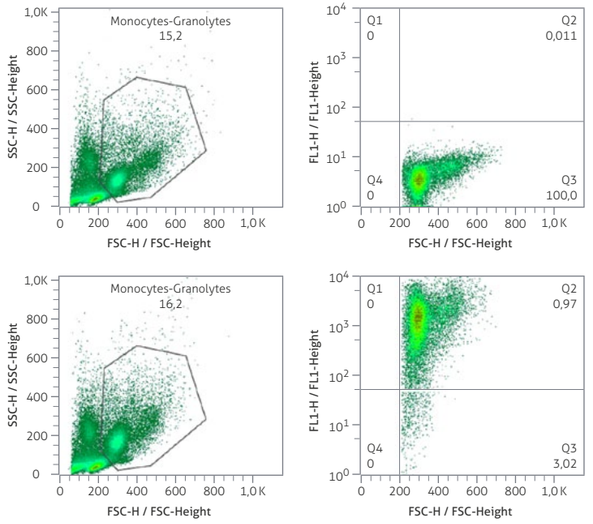
Viromer® BLUE effectively transfects human primary macrophages in a HTS setup. Reduction of EEA1 on endosomes was followed by image analysis. Z-scores are -12 and lower.

„We are absolutely delighted!"

M. Bickle, MPI CBG Dresden, Gemanny

Viromer® BLUE and GREEN – Neutral Charge of Transfection Complex results in No Aggregation and High Performance in Suspension Cells

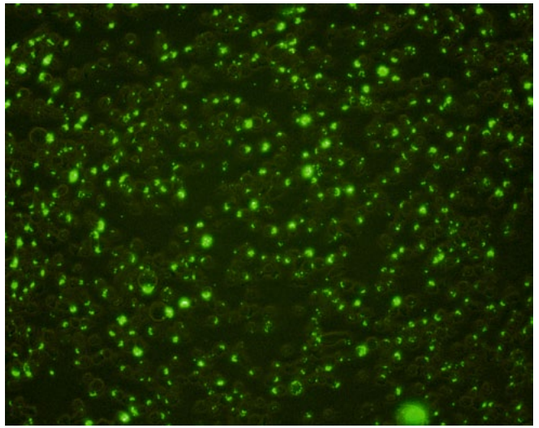
PBMCs



"I used Viromer® GREEN for transfection of PBMCs from buffy coats with a FITC labeled siRNA getting a very high transfection efficiency of 95% after 24h. In sum I like your product very much. The application is very easy and the results much better compared to Lipofectamine which I had used before."

M. Ballbach, University Tübingen, Germany

Promyoblast macrophage cell line KG-1a



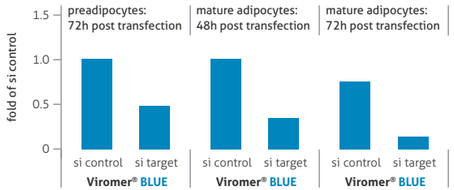
Transfection efficiency in KG-1a cells after transfection with a labeled siRNA using **Viromer® BLUE**.

"The result was exciting and encouraging. The delivery efficiency is over 90% with good fluorescence intensity."

J. Lung, C. Lung Gang Memorial Hospital, Chia-Yi, Conjoint Laboratory, Chiayi, Taiwan

Viromer® BLUE – Very Effective Knock-Down in Metabolic Cells

Adipocytes



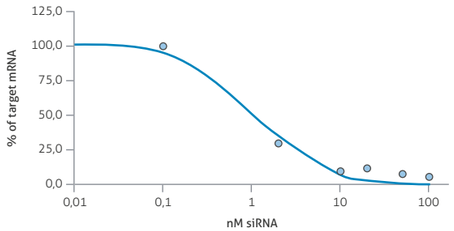
"The results show that for our cells, Viromer Blue is considerably superior to other transfection reagents."

A. Fender, University Hospital Düsseldorf, Germany

"Thanks for providing a fantastic reagent. I am looking forward to work with your chemistry."

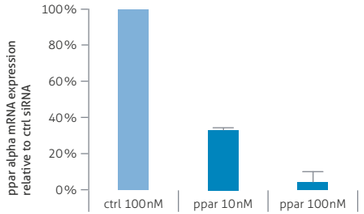
P. Hallenborg, University of Southern Denmark, Sweden

Primary skeletal myoblasts



Data generated by C. Weigert, University of Tübingen

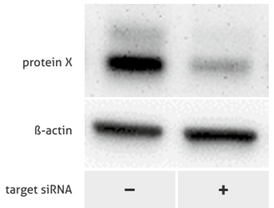
Primary hepatocytes



Freshly isolated mouse hepatocytes were transfected with **Viromer® BLUE** and ppar-alpha (blue bars) or control siRNA (ligth blue bar). 24h later prepared for RNA analysis. 100nM yielded a complete knock-down.

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

C2C12 myoblasts

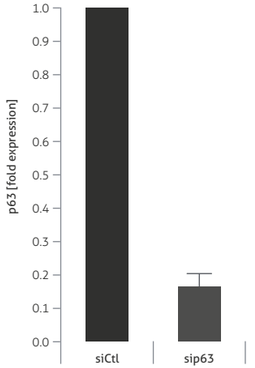


Knock-down of protein X in C2C12 myoblasts cells transfected with **Viromer® BLUE**.

Data generated by Prof. J. Hall, J. Zagalak - ETH Zurich, Institute for Pharmaceutical Science, Switzerland

Viromer® BLACK – Excellent Results in Primary Keratinocytes

Primary keratinocytes

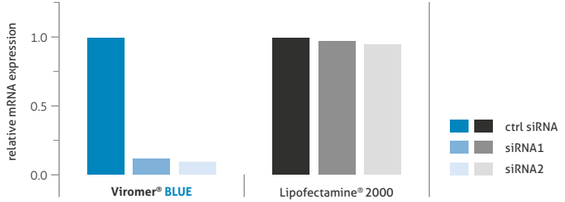


"We are very happy with the results. Now Viromer Black is a very good alternative to our standard transfection method which is associated with the use of high siRNA concentration and significant cell loss. The toxic effect with Viromers is very low and we get a very good cell yield."

University of Regensburg, Germany

Viromer® BLUE and GREEN – Strong Knock-Down in Cancer Cells

Melanoma cells



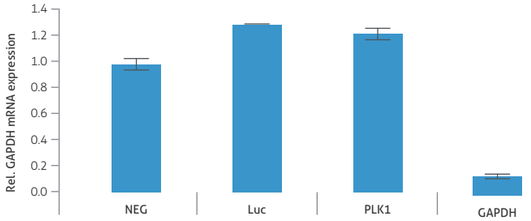
Knock-down efficiency in a melanoma cell line after transfection with **Viromer® BLUE**.

"The Viromer transfection has functioned wonderfully"

University Hospital Essen, Germany

Hs74T: Gastric carcinoma

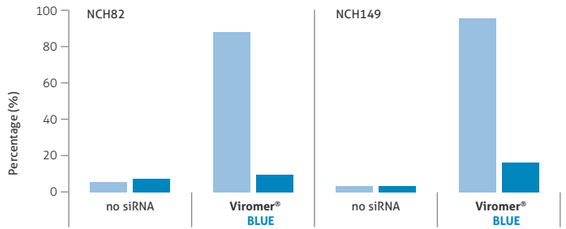
Hs746T cells were transfected with GAPDH siRNA (25nM) for 72h



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

Data generated by Gaither, Novartis, USA

Glioblastoma cells



Transfection efficiency (%, light blue bars) and cell death (%, dark blue bars) of glioblastoma cell lines after transfection of siRNAs (at 10nM) using **Viromer® BLUE**.

"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, Germany

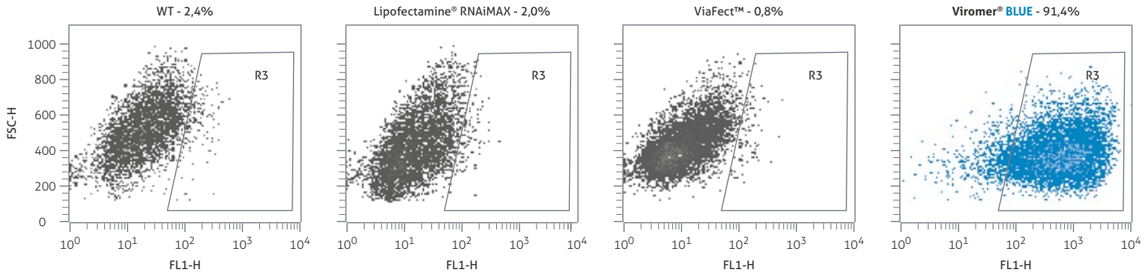
CT26: Colorectal carcinoma

Western Blot shows total reduction of Stat3 protein using its siRNA complexed to **Viromer® 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, Germany

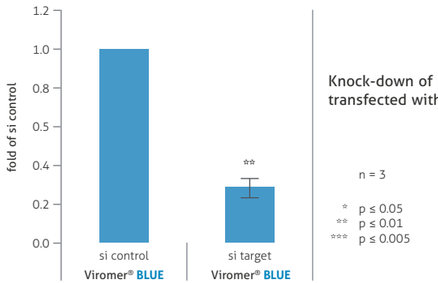
Viromer® BLUE Outperforms Standard Reagents – Superior Transfection and Knock-Down Efficiency in Primary Human Mesenchymal Stem Cells



Delivery of a fluorescein-labeled oligo in primary mesenchymal stem cells with Lipofectamine® RNAiMAX, Viafect™, and **Viromer® BLUE** in comparison to untransfected (WT) cells.

"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, University Hospital Halle, Germany



Knock-down of protein X in MSC transfected with **Viromer® BLUE.**

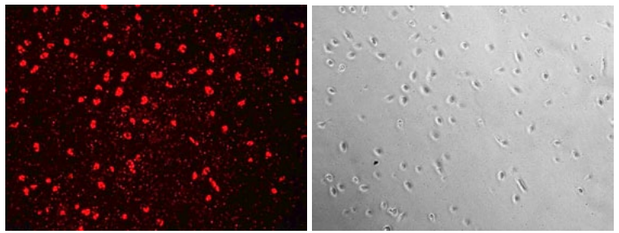
n = 3

* p ≤ 0.05
** p ≤ 0.01
*** p ≤ 0.005

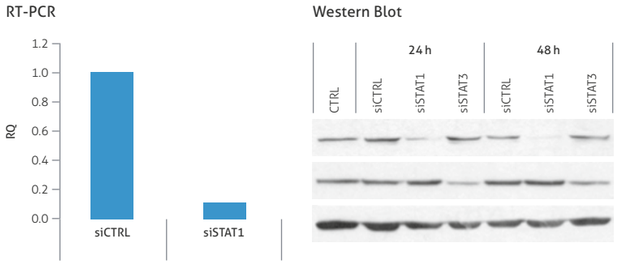
"The Viromer reagent is very appropriate to transfect MSC with siRNA. I was able to achieve a nearly 4-fold target gene knock down."

S. Werner, Institute for Physiological Chemistry, Halle, Germany

Viromer® BLUE – Strong and Specific Knock-Down in Primary Microglia Cells



Transfection efficiency in primary microglia cells transfected with siGLO-red using **Viromer® BLUE.**

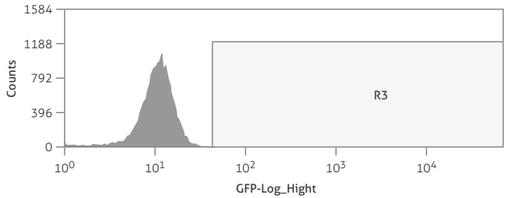


"We are very satisfied with the results. We see good silencing both at the mRNA and protein levels. Cells are viable in 80-90% under the condition that we change medium after 4h. "

M. Maleszewska, Nencki Institute of Experimental Biology Warsaw, Russia

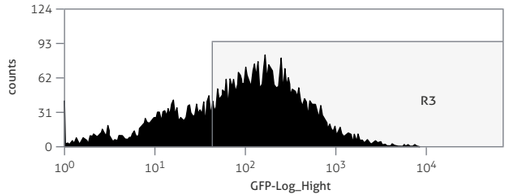
Viromer® BLACK yields 70% Transfection in Neural Stem Cells

Non transfected



Region	Count	% Hist
Total	19243	100.00
R3	14	0.07

Transfected



Region	Count	% Hist
Total	3829	100.00
R3	2655	69.34

"...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

Transfection of plasmid DNA and mRNA

VIROMER® RED

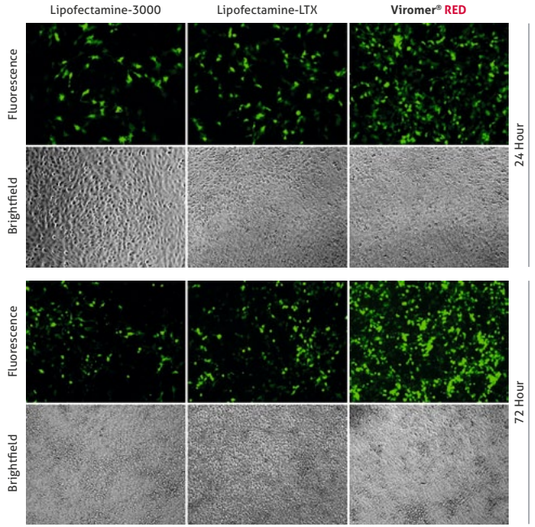
Versatile for standard and challenging cells

VIROMER® YELLOW

Selected for specific cells such as primary cardiomyocytes and hepatocytes

Viromer® RED outperforms Major Competitors on Hard-to-Transfect Cancer Cells

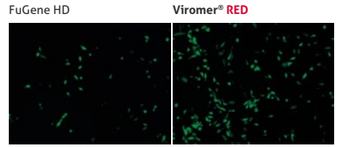
C6: Glioma cells

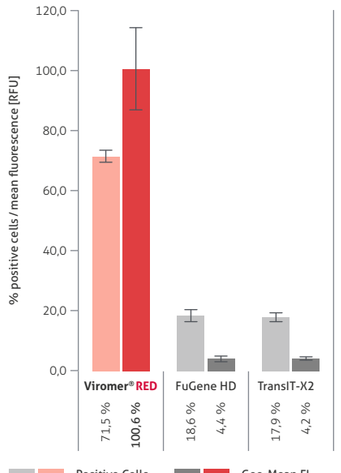


"Given the efficiency we saw, your product might be uniquely poised to address many research questions using this clinically relevant cell line. Viromer Red showed faster and better transfection efficiency in Glioma cell line as compared to Lipofectamines."

©2014 Pearse Lab, Miami Project to Cure Paralysis, Dr. S. Rao, USA

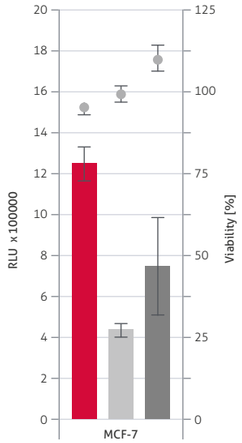
SH-SY5Y: Neuroblastoma cells





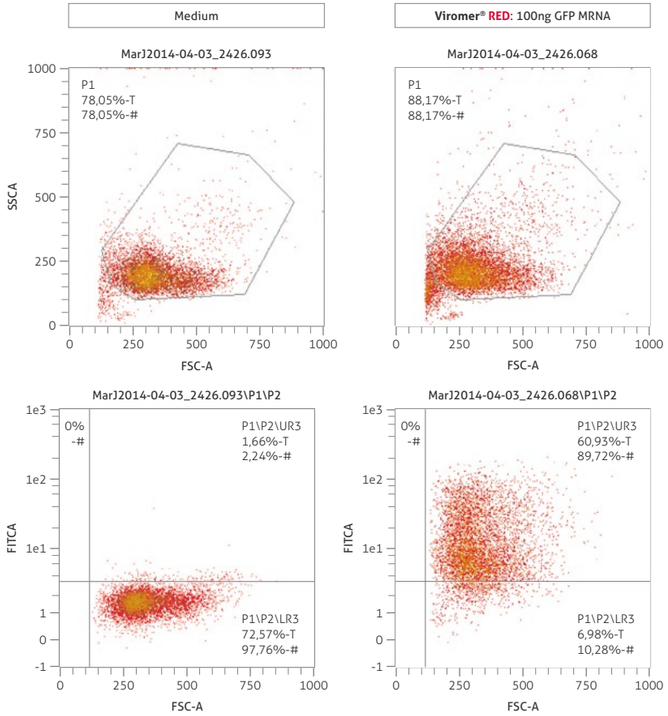
Data generated by H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany.

MCF-7 cells

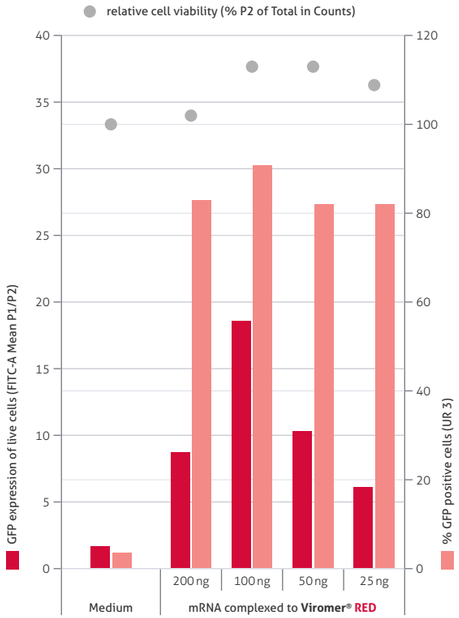


Data generated by Lipocalyx, Halle, Germany.

Viromer® RED transfects 90% of Human Primary Monocytes with mRNA



Data generated by Miltenyi Biotec, Bergisch Gladbach, Germany

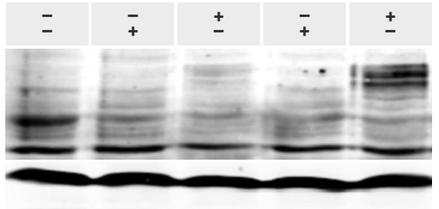


- CD14 purified monocytes from hPBMCs (Buffy Coat)
- 40.000 cells/96-well
- treatment with various doses of GFPmRNA
- GFP detection via MACSQuant

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

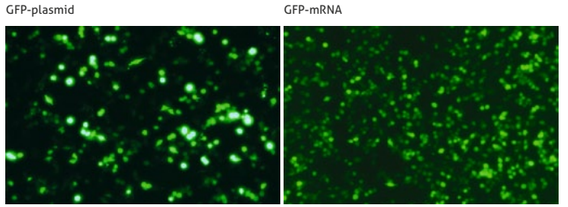
Viromer® RED – Unsurpassed Transfection of Macrophages compared to Standard Reagents

Primary human macrophages



Data generated by A. Weigert, University Hospital Frankfurt, Germany

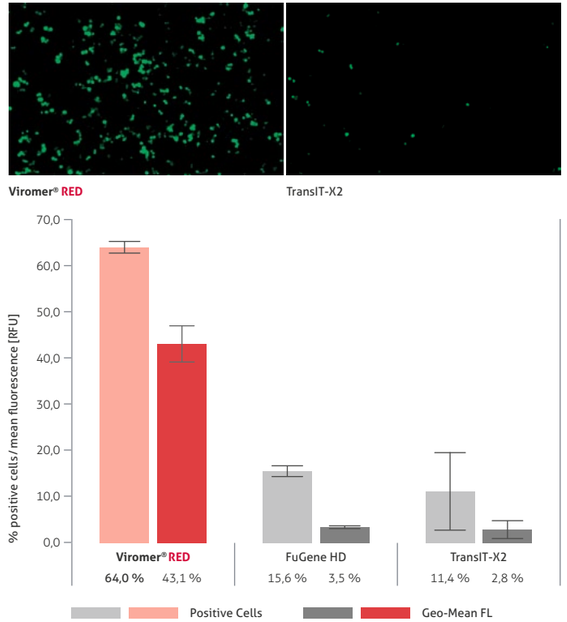
Transfection of RAW 264.7: Mouse macrophages with:



Transfection of RAW 264.7 cells with GFP-plasmid DNA and GFP-mRNA using Viromer® RED.
RAW 264.7 cells were transfected with pCMV-GFP plasmid and GFP-mRNA using **Viromer® RED**. 24h after transfection efficiency was monitored using fluorescence microscopy.

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

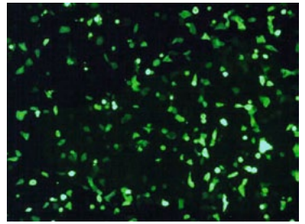
Transfection of plasmid DNA into RAW 264.7: Mouse macrophages

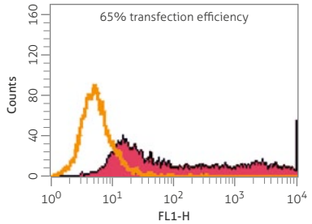


Data generated by H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany

Viromer® RED – Excellent Transfection Efficiency in Primary Keratinocytes, C2C12 myoblasts, and Pancreatic Tumor Cells

Primary keratinocytes

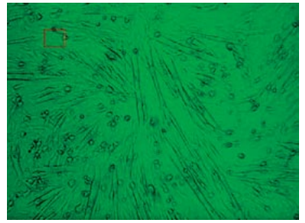




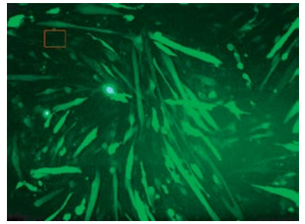
Expression of GFP in primary keratinocytes transfected with **Viromer® RED**.

Data generated by M. Podgórska; Prof. S. Smola: University Hospital Homburg/Saar, Germany.

C2C12 myoblasts



GFP-1



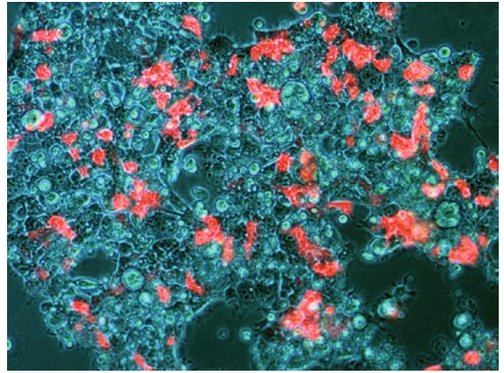
Phase contrast

Transfection efficiency after transfection of GFP-N1 plasmid into undifferentiated C2C12 cells using **Viromer® RED**.

"We have tried the Viromer on undifferentiated C2C12. The myotube remains being transfected and it works great".

Dr. CL Tse, University of Oklahoma, Health Science Center, Dept. of Physiology, USA

Primary pancreatic tumor cells

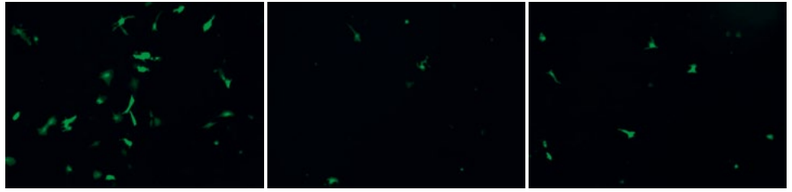


"I used the Viromer to transfect our primary pancreatic cancer cells, which are derived directly from patient tumors and they are VERY difficult to transfect with lipofectamine and infect with lentivirus. The tumor responded very well and we got very good transfection efficiency with Viromer Red."

Dr. B. Sainz, University of Madrid, Spain

Viromer® YELLOW Outperforms Standard Transfectants in Primary Cardiomyocytes and Hepatocyte Cell Line FAO

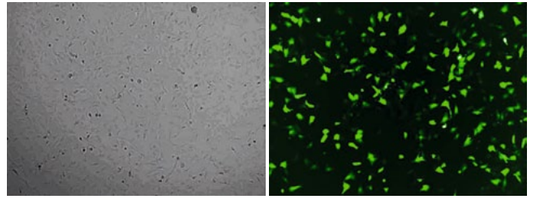
Primary cardiomyocytes



Viromer® YELLOW | **Lipofectamine® 2000** | **Lipofectamine® 3000**

"I was very happy with the results. So far I had good results with the standard protocol and lowest concentration of Viromer Yellow. I have tried incubating the myocytes with the Yellow reagent for 4 h as suggested and the results were very good."

N. Kaludercic, University of Padova, Italy

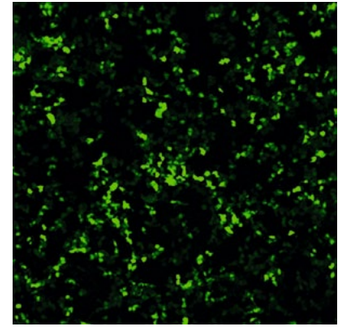


Viromer® YELLOW

"Your transfection product (Viromer YELLOW) works much better than the other reagents we have used before to transfect neonatal rat cardiomyocytes. We obtain 50% efficiency with low toxicity."

A. Castellano, University of Seville, Spain

Hepatocyte cell line FAO



Expression of GFP in rat hepatocyte cell line FAO transfected with **Viromer® YELLOW**.

"In comparison to 3 other transfection reagents Viromer YELLOW was most sensitive with highest transfection efficiency 48h post transfection."

C. Klingler, University Hospital Tübingen, Germany

Transfection using Start Positive® Controls

Start Positive® controls are preformulated, ready-to-use transfection complexes. Use them for transfection of new cell types or as reference material. Start Positive® controls for Viromer® RED/YELLOW comprise a pDNA and one mRNA sample each and facilitate comparative studies between these genetic cargoes. See results on page 17.

VIROMER® BLUE siRNA controls

- GAPDH-siRNA complexed to Viromer® BLUE
- non-targeted siRNA labeled with Cy3 complexed to Viromer® BLUE

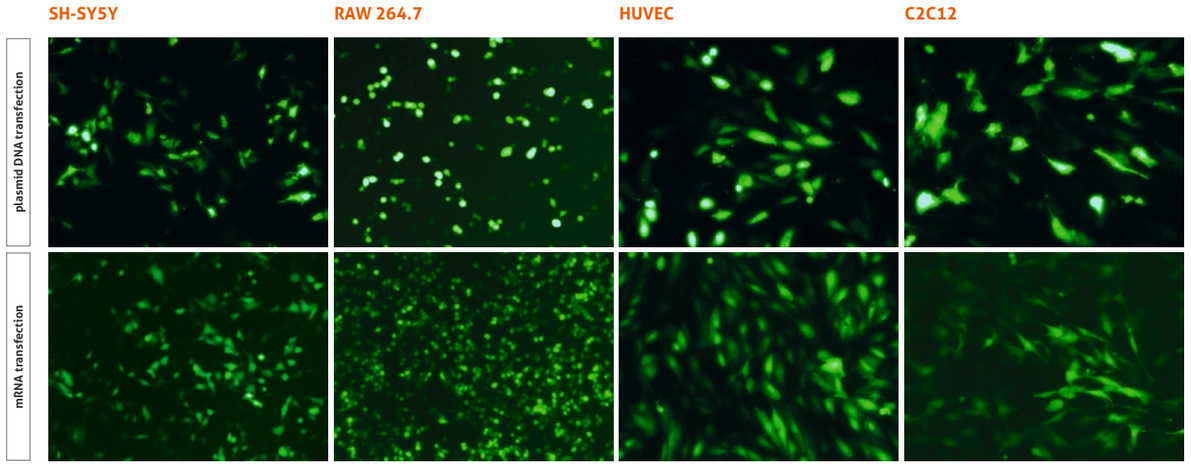
VIROMER® RED pDNA/mRNA controls

- pCMV-GFP (3.5kb) plasmid complexed to Viromer® RED
- GFP mRNA complexed to Viromer® RED

VIROMER® YELLOW pDNA/mRNA controls

- pCMV-GFP (3.5kb) plasmid complexed to Viromer® YELLOW
- GFP mRNA complexed to Viromer® YELLOW

Start Positive® Controls – Strong Transfection of Challenging Cells with plasmid DNA and mRNA

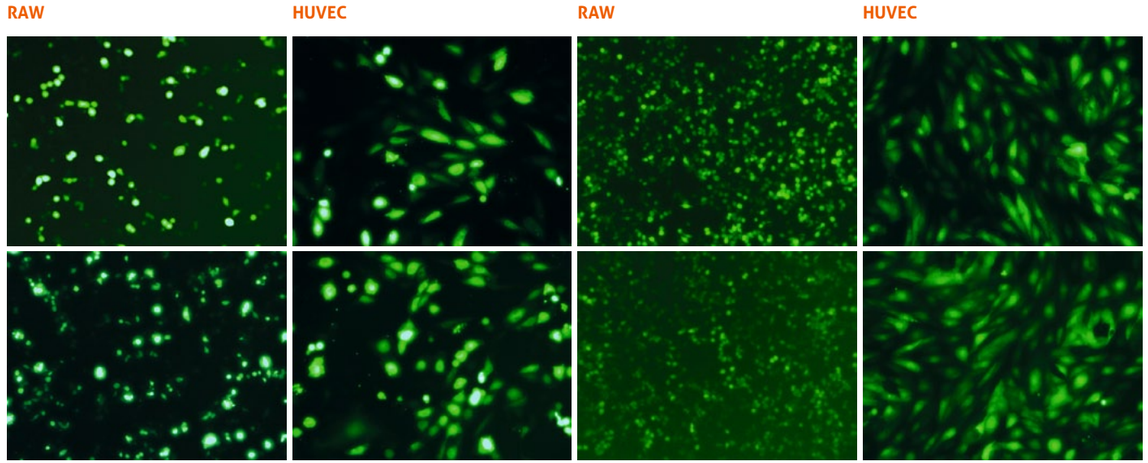


Transfection using Start Positive® Controls.

Challenging cell lines were transfected with pCMV-GFP plasmid and GFP-mRNA using Start Positive® controls of **Viromer® RED**. Transfection was monitored using fluorescence microscopy. Between pDNA and mRNA we typically observe faster, homogeneous and stronger expression from mRNA. We attribute this to the instant availability of the transcript.

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

Start Positive® Controls match Performance of Standard Viromer® Reagent



Transfection using Start Positive® Control and Viromer®.

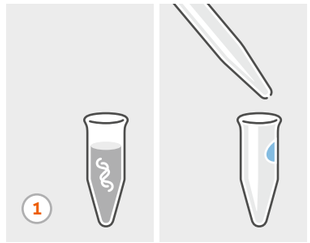
Challenging cell lines were transfected with pCMV-GFP plasmid and GFP-mRNA using Start Positive® controls or standard Viromer reagent. Transfection was monitored using fluorescence microscopy. Start Positive® controls and Viromer show equal performance.

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

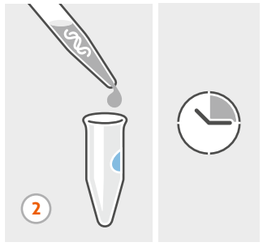
Viromer® Transfection Protocol

Straightforward and simple workflow for all plate formats

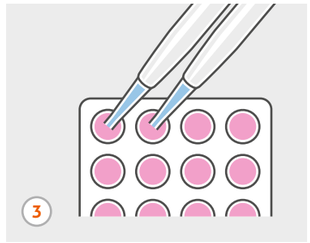
Working with Viromer®



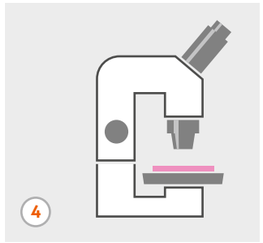
Dilute siRNA, pDNA or mRNA and provide **Viromer®** droplet



Combine dilution to Viromer droplet, mix, and wait for 15 min

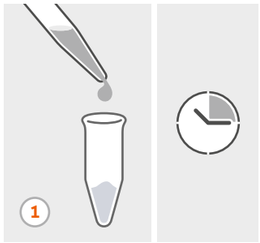


Add to your cells in fresh media

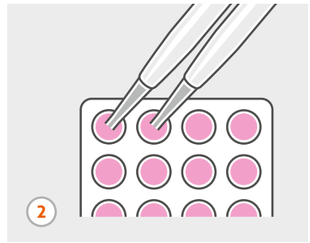


View results

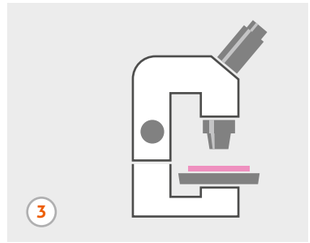
Working with Start Positive® Controls



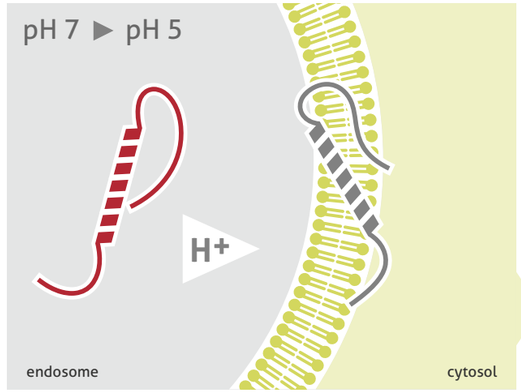
Rehydrate lyophilized complexes and wait for 10 min

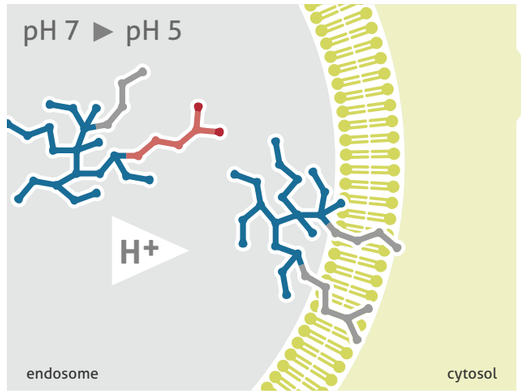


Add to your cells in fresh media



View results





Viromer®: A novel polymer based transfection reagent mimicking the viral infection process by an active endosome escape mechanism.

In influenza
the pH-sensitive fusion peptide inserts into the endosomal membrane.

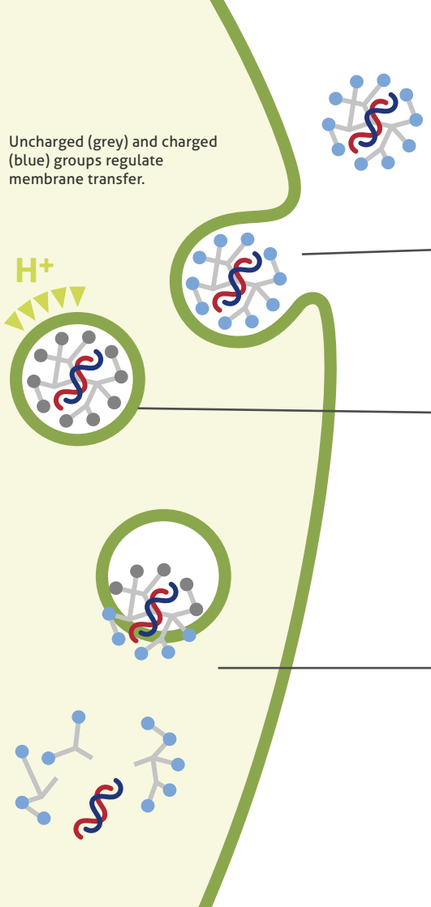
Mechanism relies on protonation of GLU, balanced by hydrophobic ALA.

Viromers
mimic the influenza mechanism, but use a polymer instead of a fusion peptide.

Fatty acids (red) resemble Influenza's GLU, alkyl (grey) are similar to ALA.

As a result, both influenza and Viromer promote an active endosome escape leading to cytosolic delivery.

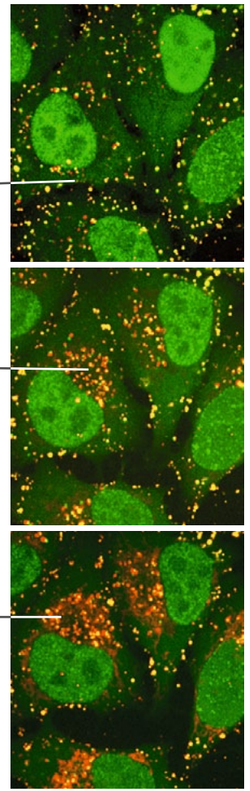
The Viromer Uptake Pathway: The Active Escape Process



1:00h – early endosome
Viromers are taken up at the cell surface.

3:00h – late endosome
Viromers accumulate near the nucleus, ongoing acidification.

4:30h – arrival in the cytosol
Discharge of siRNA from endosomes and starting diffusion.

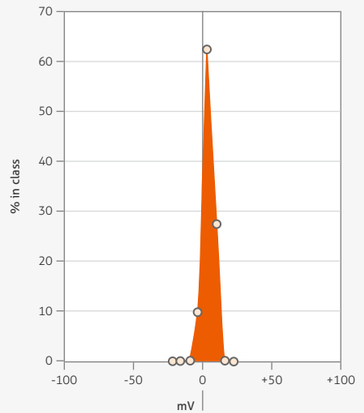


Model: HeLa, Viromer® GREEN, labelled siRNA. Data courtesy of Chromotek.

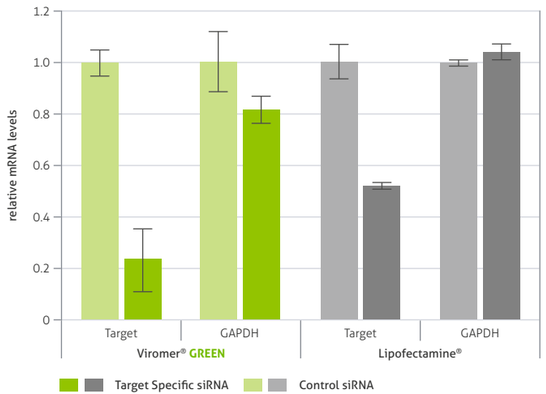
Viromer® – Neutral Charge of Transfection Complexes are proven safe and effective in Suspension or 3D Cell Cultures

Viromer® are proven superior to Lipofectamine with 3D spheroids and show advanced performance in THP-1, PBMCs, or primary microglia cells. (see page 4, 5 and 9)

Neutral Charge



3D culture of cancer stem cells (mammospheres)



Knockdown of target and GAPDH control in cancer mammospheres.

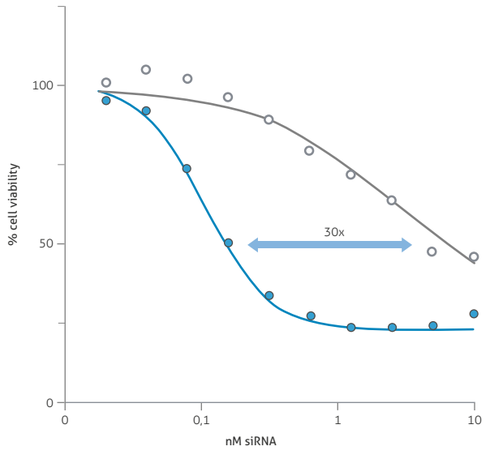
"And thank you very much for letting us test the Viromer system, we were satisfied with the efficiency of knockdown."

J. Holland, MDC Berlin

Viromer® – Safe Transfection with a Wide Working Range

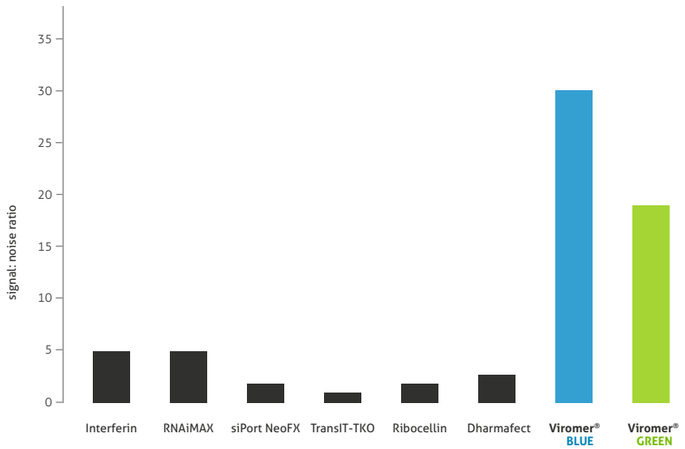
The outcome of RNAi experiments depends on the ratio between the siRNA signal and any unspecific noise. Viromers® achieve signal: noise ratios between 20 and 30, much better than six leading competitors.

Viromer® BLUE phenotypic assay



Very low background in a phenotypic assay. Cell cycle of HeLa was arrested using PLK-1 siRNA (filled symbols, the signal) or a control siRNA (open symbols, the noise). For **Viromer® BLUE**, the EC50 values differ by a factor of 30.

Signal: noise ratios of transfectants



Signal: noise ratio for various transfectants. Signal: noise is defined as the ratio of EC50 values for the active and control siRNA.

Products & Ordering

Viromer® for siRNA/miRNA applications	Product Number	Transfections in 24-well
Viromer® **BLUE**	VB-01LB-00	50
Viromer® **BLUE**	VB-01LB-01	500
Viromer® **BLUE**	VB-01LB-03	3 x 500
Viromer® **BLUE** GAPDH/CY3 siRNA controls	VB-siBUNDLE-01	
Viromer® **GREEN**	VG-01LB-00	50
Viromer® **GREEN**	VG-01LB-01	500
Viromer® **GREEN**	VG-01LB-03	3 x 500
Viromer® **BLACK**	VBk-01LB-00	80
Viromer® **BLACK**	VBk-01LB-01	500
Viromer® **BLACK**	VBk-01LB-03	3 x 500

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Viromer® for plasmid/mRNA applications	Product Number	Transfections in 24-well
Viromer® **RED**	VR-01LB-00	50
Viromer® **RED**	VR-01LB-01	600
Viromer® **RED**	VR-01LB-03	3 x 600
Viromer® **RED** pDNA-/mRNA-GFP controls	VR-BUNDLE-01	
Viromer® **YELLOW**	VY-01LB-00	50
Viromer® **YELLOW**	VY-01LB-01	1 x 600
Viromer® **YELLOW**	VY-01LB-03	3 x 600
Viromer® **YELLOW** pDNA-/mRNA-GFP controls	VY-BUNDLE-01	

Webshop: www.viromer-transfection.com

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VIROMER® **BLACK**

VIROMER® **RED**

VIROMER® **YELLOW**