THE VIROMER® FACTBOOK

Transfection of siRNA, mRNA and plasmids

2015/2016
Welcome to Viromer® Transfection Reagents! We provide:

- High transfection efficiency
- Great safety
- Easy and fast transfection with consistent results

 Due to an active escape of Viromer® complexes from the endosome
Because Viromer® complexes are non-charged, gentle on cells and compatible with serum and antibiotics
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.

Due to an active escape of Viromer® complexes from the endosome because Viromer® complexes are non-charged, gentle on cells and compatible with serum and antibiotics, ascribed to straightforward protocol including initial optimization.

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

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.

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

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

“We are absolutely delighted!”
M. Bickle, MPI CBG Dresden, Germany

“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

“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

Viromer® BLACK – Excellent Results in Primary Keratinocytes

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

**Adipocytes**

- Mature preadipocytes
- Mature adipocytes
- Mature adipocytes

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<th>Treatment</th>
<th>Protein Expression</th>
<th>Relative mRNA Expression</th>
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<tr>
<td>10nM ppar alpha sirNa</td>
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**Primary hepatocytes**

- Fetal post transfection
- 7th post transfection

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**Primary keratinocytes**

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</table>

**Freshly isolated mouse hepatocytes were transfected with Viromer® BLUE and ppar-alpha (blue bars) or control sirRNA (light blue bar). 4h later prepared for RNA analysis. 100nM yielded a complete knock-down.**

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

**Viromer® bluE – Very Effective Knock-Down in Metabolic Cells**

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

**Viromer® BLACK – Excellent Results in Primary Keratinocytes**

**C2C12 myoblasts**

- protein X
- ß-actin

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

**Data generated by C. Weigert, University of Tübingen**

**Primary skeletal myoblasts**

**Data generated by Dr. S. Vaquet, University of Tübingen**

**Viromer® In METABOLIC RESEARCH**

**Viromer® In ONCOLOGY RESEARCH**

**Globlastoma cells**

**CT26: Colon cancer**

**Hs74T: Gastric carcinoma**

**Western blot shows total reduction of Stat3 protein using its sirRNA complexed to Viromer® GREEN. Scrambled control sirRNA has no effect on Stat3 protein levels.**

**Data generated byoa, Novartis, USA**

**Viromer® Greens**: Efficient and safe transfected Hs746T cells. Expression of GAPDH mRNA was reduced by 90% without any signs of toxicity.

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

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

**A. Fender, University Hospital Düsseldorf, Germany**

**“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**

**“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 sirRNA concentrations and significant cell loss. The knock-down effect with Viromer is very low and we got a very good cell yield.”**

**University of Regensburg, Germany**

**“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**

**“The Viromer transfection has functioned wonderfully”**

**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**

**Viromer® bluE and BLACK – Excellent Results in Primary Keratinocytes**

**Viromer® bluE and GREEn – Strong Knock-Down in Cancer Cells**

**Glioblastoma cells**

**Transfection efficiency (% light blue bars) and cell death (% dark blue bars) of glioblastoma cell lines after transfection of sirRNAs (at 15nM) 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.”**

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**University of Regensburg, Germany**

**Data generated by Gaither, Novartis, USA”**

**Primary skeletal myoblasts**

**Data generated by C. Weigert, University of Tübingen”**

**Viromer® bluE – Very Effective Knock-Down in Metabolic Cells”**

**Viromer® BLACK – Excellent Results in Primary Keratinocytes”**

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

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**F. Greten, Georg-Speyer-Haus Frankfurt, Germany”**
Viromer® BLUE Outperforms Standard Reagents – Superior Transfection and Knock-Down Efficiency in Primary Human Mesenchymal Stem 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

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

Viromer® BLACK yields 70% Transfection in Neural Stem Cells

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

C6: Glioma cells

Viromer® RED

fuGène HD

TransIT-X2

Viability [%]

Lipofectamine® 2000

Lipofectamine® LTX

Viromer® RED

Luciferase Activity [RLU]

“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

Viromer® RED

Versatile for standard and challenging cells

Selected for specific cells such as primary cardiomyocytes and hepatocytes

Transfection of plasmid DNA and mRNA

Viromer® YELLOW

SH-SY5Y: Neuroblastoma cells

MCF-7 cells

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

Data generated by Lipocalyx, Halle, Germany.

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

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

Primary human macrophages

Transfection of plasmid DNA into RAW 264.7: Mouse macrophages

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

Transfection of RAW 264.7 cells with GfP-plasmid and GfP-mRNA complexed to Viromer® RED:

CD14 purified monocytes from PBMCs (Buffy Coat)

40,000 cells/96-well

Treatment with various doses of GfP-mRNA

GFP detection via MACSQuant

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

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

C2C12 myoblasts

Primary pancreatic tumor cells

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

Primary cardiomyocytes

Hepatocyte cell line FAO

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

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

"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

"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

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

"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

"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

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

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

Viromer® RED

"60% transfection efficiency"

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

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

Viromer® RED

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."

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

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

**VIROMER® YELLOW**
- 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**

SH-SY5Y

RAW 264.7

HUVEC

C2C12

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 H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany.
Start Positive® Controls match Performance of Standard Viromer® Reagent

Viromer® Transfection Protocol
Straightforward and simple workflow for all plate formats

Working with Viromer®
1. Dilute siRNA, pDNA or mRNA and provide Viromer® droplet.
2. Combine dilution to Viromer droplet, mix, and wait for 15 min.
3. Rehydrate lyophilized complexes and wait for 10 min.
4. Add to your cells in fresh media.
5. View results.

Working with Start Positive® Controls
1. Dilute siRNA, pDNA or mRNA and provide Viromer® droplet.
2. Combine dilution to Viromer droplet, mix, and wait for 15 min.
3. Rehydrate lyophilized complexes and wait for 10 min.
4. Add to your cells in fresh media.
5. View results.

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

Start Positive® Controls match Performance of Standard Viromer® Reagent

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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.
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)

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

J. Holland, MDC Berlin

Neutral Charge

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.

"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® BLUE phenotypic assay

Viromer® BLUE phenotypic assay

Signal: noise ratios of transfectants

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 ratio for various transfectants. Signal: noise is defined as the ratio of EC50 values for the active and control siRNA.
Viromer® for siRNA/miRNA applications

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www.mabio.com

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www.interchim.com

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Biozym Scientific GmbH (DE)
www.biozym.com

Italy
Voden Medical Instruments S.p.a.
www.vodenmedical.com

Korea
Dong In Biotech CO., Ltd.
www.donginbio.com

Poland
BLIRT S.A.
www.dnagdansk.com

United Kingdom & Ireland
Cambridge Bioscience Ltd.
www.bioscience.co.uk

USA
OriGene Technologies, Inc.
www.origene.com

Australia & New Zealand
TrendBio Pty Ltd
www.trendbio.com.au

Austria
Biozym Biotech Trading GmbH (AUT)
www.biozym.com

Brasilia
Sinapse Biotechnologia
www.sinapsebiotecnologia.com

China
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www.mabio.com

France
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www.interchim.com

Germany
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www.biozym.com

Italy
Voden Medical Instruments S.p.a.
www.vodenmedical.com

Japan
Nakamura Scientific Instruments Industry Co., Ltd.
www.nmkkk.co.jp

Korea
Dong In Biotech CO., Ltd.
www.donginbio.com

Poland
BLIRT S.A.
www.dnagdansk.com

United Kingdom & Ireland
Cambridge Bioscience Ltd.
www.bioscience.co.uk

USA
OriGene Technologies, Inc.
www.origene.com
www.viromer-transfection.com

Lipocalyx GmbH   Lipocalyx US
Weinbergweg 23   1 Jill Ct. Building 16 Unit 10
06120 Halle     Hillsborough, NJ 08844
Germany         USA