

Technical Bulletin

MISSION® Lentiviral Transduction Particles

Catalog Number SHCLNV

Product Description

Small interfering RNAs (siRNAs) processed from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) in mammalian cells. The MISSION product line is a viral vector-based RNAi library against annotated mouse and human genes. shRNAs that are processed into siRNAs intracellularly are expressed from amphotropic lentivirus particles, allowing screening in a wide range of mammalian cell lines. MISSION shRNA clones permit rapid, cost-efficient loss-of-function and genetic interaction screens.

The Lentiviral Transduction Particles are produced from a library of sequence-verified lentiviral plasmid vectors for mouse and human genes. The TRC1 and TRC1.5 libraries consist of sequence-verified shRNAs cloned into the pLKO.1-puro vector (see Figure 1). The TRC2 library consists of sequence-verified shRNAs in the TRC2-pLKO-puro vector (see Figure 2). The TRC2 vector has a single additional element in comparison to the TRC1 vector. This is the WPRE,¹ or the Woodchuck Hepatitis Post-Transcriptional Regulatory Element. WPRE allows for enhanced expression of transgenes delivered by lentiviral vectors.²

A number of individual shRNAs designed using a proprietary algorithm are available for each gene. We recommend purchasing multiple individual constructs (the recommended number is listed on each clone ordering page) targeting different regions of the gene sequence.

A range of knockdown efficiencies can be expected when using multiple clones. This allows one to examine the effect of loss of gene function over a large series of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based particles permit efficient infection and integration of the specific shRNA construct into differentiated and non-dividing cells, such as neurons and dendritic cells,³ overcoming low transfection and integration difficulties when using these cell lines. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.⁴⁻⁵

In addition, the lentiviral transduction particles are pseudotyped with an envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G), allowing transduction of a wide variety of mammalian cells

including primary and embryonic stem cells.⁶ The lentiviral particles are titered via a p24 antigen ELISA assay and pg/ml of p24 are then converted to Viral particles (VP) per ml using a conversion factor.

Components

The individual constructs are provided in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin. There are several available options for volume, titer, and vector backbones.

Volumes available
▪ 0.1 mL
▪ 0.2 mL
▪ 1.0 mL
▪ 2.0 mL
▪ 5.0 mL
▪ 10.0 mL

Titers available
▪ 10^6 VP/ml
▪ 10^7 VP/ml
▪ 10^8 VP/ml
▪ 10^9 VP/ml

Note: Not all volume and titer combinations are available.

	10^6 VP/ml	10^7 VP/ml	10^8 VP/ml	10^9 VP/ml
0.1 mL	NA	x	x	x
0.2 mL	x	x	x	x
1.0 mL	x	x	x	x
2.0 mL	x	x	x	x
5.0 mL	x	x	x	NA
10.0 mL	x	x	x	NA

Vector backbones available:

- pLKO.1 (refers to the TRC version listed for the particular clone)
- pLKO.1-CMV-Neo
- pLKO.1-hPGK-Neo
- pLKO.1-CMV-tGFP
- pLKO.1-hPGK-Neo-CMV-tGFP
- pLKO.1-hPGK-Puro-CMV-tGFP

Note: The customizable vectors are based on the TRC1 vector.

Orders of 25 or fewer clones are provided in individual vials. Each vial contains a unique one-dimensional barcode label that can be read using a corresponding reader. A printed value corresponding to The RNAi Consortium (TRC) clone number is also provided on each tube. Orders of >25 clones are provided in a 96-well plate with a one-dimensional barcode label on the plate. 96-well plates are provided with a USB containing plate map positions.

Precautions and Disclaimer

For R&D use only. Not for drug, household or other uses.

Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.⁷ Follow all published RGL-2 guidelines for handling and waste decontamination. Also, use extra caution when using lentiviral transduction particles that express shRNA-targeting genes involved in cell cycle control, e.g., tumor suppressor genes.

Storage/Stability

All components are stable for at least six months after receipt when stored at -70°C . Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer.



Related Products

- Hexadimethrine Bromide, Catalog Number H9268
- Puromycin dihydrochloride, Ready Made Solution, 10 mg/ml in H₂O, Catalog Number P9620
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- PCR Reagents, please visit sigma-aldrich.com/pcr
- Prestige Antibodies, please visit sigma-aldrich.com/prestige

Procedure for the Use of MISSION Lentiviral Transduction Particles

Day 1

Plate the mammalian cell line of choice in complete medium 24 hours prior to transduction. Take into account the length of time that the cells will be cultured prior to performing RNAi analysis when determining plating density. Typically, cells are transduced at 50-80% confluency.

Day 2

Thaw the lentiviral stock slowly on ice. Gently spin down material in tubes before opening. Add hexadimethrine bromide (the chemical equivalent of Polybrene) to the cells at a final concentration of 8 µg/ml.

Note: Hexadimethrine bromide enhances transduction of most cell types. However, some cells, such as primary neurons, are sensitive to hexadimethrine bromide. When using sensitive cells, do not add the hexadimethrine bromide and the cells should still be transduced.

Following addition of hexadimethrine bromide, gently swirl the plate to mix. Add the appropriate amount of viral particles at a suitable multiplicity of infection (MOI) and swirl the plate gently to mix. Incubate the cell-viral particle mixture at 37 °C overnight.

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.

To calculate MOI:

(Total number of cells per well) × (Desired MOI) =

Total viral particles (VP) needed

$$\frac{\text{(Total VP needed)}}{\text{(VP/ml reported on C of A)}} =$$

Total ml of lentiviral particles to add to each well

Notes

- When transducing a lentiviral construct into a cell line for the first time, it is recommended that a range of MOIs (0.5 – 20) be used to find the optimal degree of target knockdown.
- When overnight incubation presents a toxicity concern, cells may be incubated for as little as 4 hours before changing the medium.

Day 3

Remove the viral particle-containing medium and replace it with fresh, pre-warmed complete culture medium.

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Day 4

Perform one of the following based on whether the transduction experiment is transient or stable:

- a. For transient expression experiments - Harvest the cells and assay for interference of the target gene. This can be done by a variety of methods such as qRT-PCR or Western blot.
- b. For stable expression experiments - Remove the medium and replace it with fresh, complete medium that contains the appropriate amount of puromycin for selection of transduced cells. Proceed to Day 5.

Note: When the appropriate concentration of puromycin for a specific cell type is unknown, perform a kill curve experiment. Typically, puromycin concentrations ranging from 0.5 – 10 µg/ml are sufficient to kill most untransduced mammalian cell lines.

Puromycin titration (kill curve) should be performed when working with a new cell type.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 µL fresh media.
2. The next day add 0.5 – 10 µg/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3 – 14 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the media containing puromycin every 3 days. The minimum concentration of puromycin that causes complete cell death after the desired time should be used for that cell type and experiment.

Note: Excess puromycin can cause many undesired phenotypic responses in most cell types.

Day 5 and forward

Replace medium with fresh, puromycin-containing medium every 3 – 4 days until resistant colonies can be identified (generally, 10 – 12 days after selection). Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.

Note: Due to the random integration of the lentivirus into the genome, varying levels of target gene knockdown may be seen from different puromycin-resistant clones. Testing a number of puromycin-resistant clones will allow a determination of which one provides the optimal degree of gene knockdown.

Troubleshooting Guide

Problem	Cause	Solution
Low levels of target gene knockdown due to low transduction efficiency	Hexadimethrine bromide not included during transduction	Transduce in the presence of hexadimethrine bromide.
	Non-dividing cell type used	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag®.
	MOI is too low	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag.
	Cells were harvested and assayed too soon after transduction	The shRNA must be permitted to accumulate in cells. Harvest 48-72 hours after transduction. Alternatively, knockdown results may be improved by placing cells under puromycin selection because untransduced cells will be killed.
No gene knockdown is observed	Viral stock stored incorrectly	Store stocks at -70 °C. Do not freeze/thaw more than 3 times.
	MOI is too low	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag.
Cytotoxic effects observed after transduction	Target gene is essential for cell viability	Be sure that target gene is not essential for cell growth or viability.
	Hexadimethrine bromide was used during transduction	Be sure that cells are not sensitive to hexadimethrine bromide. Omit the hexadimethrine bromide during the transduction.
	Too much puromycin was used for selection	Determine the puromycin sensitivity of the cells by performing a kill curve and use the minimum concentration required to kill the untransduced cells.

Control Selection Table

Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.⁸ Additional controls are available. For a complete list, please visit:

<http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/trc-shrna-products/shrna-controls.html>

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will provide a reference point for comparing all other samples.
Negative Control: Transduction with empty viral particles, containing no shRNA insert	MISSION pLKO.1-puro Empty Vector Control Transduction Particles, Catalog Number SHC001V The empty viral particles, produced from pLKO.1-puro, are a useful negative control that will not activate the RNAi pathway because they do not contain an shRNA insert. It will allow for observation of cellular effects of the transduction process. Cells transduced with the empty viral particles provide a useful reference point for comparing specific knockdown.
Negative Control: Transduction with non-targeting shRNA	MISSION pLKO.1-puro Non-mammalian shRNA Control Transduction Particles, Catalog Number SHC002V This non-targeting shRNA is a useful negative control that will activate RISC and the RNAi pathway, but does not target any human or mouse genes. The short-hairpin sequence contains 5 base pair mismatches to any known human or mouse gene. This allows for examination of the effects of shRNA transduction on gene expression. Cells infected with the non-target shRNA will also provide a useful reference for interpretation of knockdown.
Positive Control: Transduction with positive reporter viral particles	MISSION pLKO.1-puro-CMV-TurboGFP™ Positive Control Transduction Particles, Catalog Number SHC003V This is a useful positive control for measuring transduction efficiency and optimizing shRNA delivery. The TurboGFP Control transduction particles are produced from the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Transfection of this control provides fast visual confirmation of successful transduction.
Positive Control: Transduction with shRNA targeting reporter vector	MISSION pLKO.1-puro TurboGFP shRNA Control Transduction Particles, Catalog Number SHC004V The TurboGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1-puro vector containing shRNA that targets TurboGFP (Catalog Number SHC004). These particles can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this shRNA targets TurboGFP, and it does not target any human or mouse genes, it can also be used as a negative non-target control in shRNA experiments.

Cell Type Table

The cell types listed below have been successfully infected by pLKO.1-puro based shRNA constructs. Optimal conditions will need to be determined for your experimental needs. For the most updated cell line list, and some guidelines for conditions, please visit:

<http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/getting-started.html>

Cell lines, human	Cell Type
HEK293	embryonic kidney cells
HeLa	cervical adenocarcinoma
A549	lung adenocarcinoma
H1299	lung carcinoma
HT29-D4	colon carcinoma
HepG2	hepatocellular carcinoma
HCT116	colon carcinoma
MCF7	breast carcinoma
MCF10A	breast carcinoma
Panc-1	pancreatic epithelioid carcinoma
PC3	prostate carcinoma
DU145	prostate carcinoma
A431	epidermal carcinoma
THP1	monocytic
RAW264.7	macrophage
SH-SY5Y	brain neuroblastoma
HCN-1A	brain cortical neuron

Cell lines, human	Cell Type
SupT1	T-cells
BJ-TERT	diploid fibroblasts
Cell lines, mouse	Cell Type
NIH3T3	fibroblast
Primary cells, human	Cell Type
astrocytes	normal
C3H10T1/2	mesenchymal
dendritic	immature dendritic
T-cells	lymphocytes
epithelial	prostate
fibroblasts	primary mammary
Primary cells, other species	Cell Type
ECS	mouse embryonic stem cells
fibroblasts	mouse embryonic fibroblasts
MC3T3-E1	mouse bone marrow derived
molar mesenchymal	mouse embryonic mesenchymal
cardiomyocytes	rat neonatal cardiomyocytes

References

1. Donello, J.E., *et al.*, Woodchuck hepatitis virus contains a tripartite posttranscriptional regulatory element. *J Virol.*, 72, 5085-5092 (1998).
2. Zufferey, R., *et al.*, Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J Virol.*, 73, 2886-2892 (1999).
3. Stewart, S.A., *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, 9, 493-501 (2003).
4. Zufferey, R., *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* 15, 871-885 (1997).
5. Zufferey, R., *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J Virol.*, 72, 9873-9880 (1998).

6. Burns, J.C., *et al.*, Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors: Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA*, 90, 8033-8037 (1993).
7. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (<http://www4.od.nih.gov/oba>).
8. Whither RNAi? *Nature Cell Biology*, 5, 489-490 (2003).

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