

Product Information

MISSION® shRNA Mouse Gene Family Sets, Lentiviral Particles

Catalog Numbers **SM0131**, **SM0431**, and **SM2031**

Storage Temperature -70 °C

TECHNICAL BULLETIN

Product Description

Small interfering RNAs (siRNAs) generated from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) for extended periods of time in mammalian cells. The MISSION® product line is a viral-vector-based RNAi library against annotated mouse and human genes. MISSION shRNAs are expressed intracellularly after transduction with amphotropic lentivirus particles, allowing screening in a wide range of mammalian cell lines. In these cell lines, MISSION shRNA clones permit rapid, cost efficient loss-of-function and genetic interaction screens. These murine gene family sets were designed specifically for the most common mammalian model organism, the mouse. A collection of reviews that highlight the importance of each gene family set is included.

The MISSION shRNA Gene Family Sets allow for high throughput loss-of-function and genetic interaction screens. This set consists of VSV-G pseudotyped lentiviral particles. Each MISSION shRNA clone is constructed within the lentivirus plasmid vector pLKO.1-puro.¹ Each gene target set consists of 3 or more constructs that have been designed against each target gene using a proprietary algorithm. Therefore, a range of knockdown efficiency, with at least one construct from each gene set being >70%, can be expected when using these 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.

The lentiviral transduction particles are produced from a library of sequence-verified shRNA lentiviral plasmid vectors. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK 293T) by co-transfection with compatible packaging plasmids.^{2,3} 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.⁴

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,⁵ overcoming low transfection and integration difficulties when using these cell lines.

Please see the **Mouse Cell Type Table** for those cell types that have been successfully infected by pLKO.1-puro based shRNA constructs.

The lentiviral transduction particles are titered via a p24 antigen ELISA and pg/mL of p24 are then converted to transducing units per mL using a conversion factor. The conversion can be viewed at: www.tronolab.com.

Components

Each individual construct is provided as 4 x 50 µL of frozen stock containing 10⁶ lentiviral transducing particles per mL in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin. 10% of the clones in a set are titered via an ELISA p24 assay for quality control. Fully titered sets are available on a custom basis; contact RNAi@sial.com for more information.

Lentivirus sets are packaged in a 96-well plate and labeled with 2-D barcodes for simple plate identification. A CD contains detailed clone position, 2-D barcode reference, RefSeq, locus link, gene description, gene symbol, clone ID, and hairpin sequence.

The hairpin sequence and other unique clone information may be obtained by searching the MISSION database at www.sigma.com/yfg using: RefSeq accession numbers, e.g., NM_027088, unique clone identification numbers, e.g., NM_027088.1-989s1c1, or TRC numbers, e.g., TRCN0000030720.

Equipment and reagents required but not provided

- Hexadimethrine bromide, Catalog Number H9268
- Puromycin 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
- 96-well cell culture treated plates
- Mammalian cells to be transduced
- Primers, probes, and PCR mix for qRT-PCR
- Cell-based, enzymatic, or array based assay for phenotypic assay

Catalog Number	Gene Family Set	Gene Count *	Clone Count *	Average Number Clones/Gene *
SM0131	Kinases	618	3916	6.3
SM0431	Phosphatases	290	1734	6.0
SM2031	Tyrosine Kinases	133	850	6.4

*The MISSION production and bio-informatics team constantly reviews and quality controls clones available for a gene family set. These numbers are very close to the actual number that will be shipped, but each researcher will receive a final plate map indicating the location and exact TRCN clone numbers.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.⁶ 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.

Preparation Instructions

- Prepare mammalian cell cultures so that they are growing exponentially and are no more than 70–80% confluent before transduction.
- Prepare a stock solution of hexadimethrine bromide, the chemical equivalent of Polybrene, at 2 mg/mL in water.

Storage/Stability

All components are guaranteed to be stable for at least six months after receipt when stored at –70 °C. Avoid repeated freeze/thaw cycles, as this will severely reduce transduction efficiency.

Procedure

The following protocol has been developed for high-content screening in 96-well plates with stable selection through puromycin.

Day 1

1. Add 1.6×10^4 cells in fresh medium to the number of wells needed for each construct in a 96-well plate. Duplicate or triplicate wells for each lentiviral construct and control should be used.
2. Incubate 18–20 hours at 37 °C in a humidified incubator in an atmosphere of 5–7% CO₂.

Note: The growth rates of cells vary greatly. Adjust the number of cells plated to accommodate a confluency of 70% upon transduction. Also account for the length of time the cells will be growing before downstream analysis when determining the plating density.

Day 2

1. Remove the medium from wells. To each well add 110 µL of medium and hexadimethrine bromide to a final concentration of 8 µg/mL. Gently swirl the plate to mix.

Note: Hexadimethrine bromide enhances transduction of most cell types. Some cells, like primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide to these types of cells. If working with a cell type for the first time, a hexadimethrine control only well should be used to determine cell sensitivity.

2. Add 2–15 µL of lentiviral particles to appropriate wells. Gently swirl the plate to mix. Incubate 18–20 hours at 37 °C in a humidified incubator in an atmosphere of 5–7% CO₂. Cells may be incubated for as little as 4 hours before changing the medium containing lentiviral particles. Overnight incubation may be avoided when toxicity of the lentiviral particles is a concern.

Note: When transducing a lentiviral construct into a cell line for the first time, a range of volume, or Multiplicity of Infection (MOI), should be tested. 2, 5, 10, and 15 µL of lentiviral particles per 1.6×10^4 cells or MOIs of 0.5, 1, 2, and 5 should be used to determine the optimal transduction efficiency and knockdown for each cell line. Transduction efficiency can be optimized using the TurboGFP™ Control Transduction Particles (Catalog Number SHC003V).

Multiplicity of Infection (MOI):

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:

$$(\text{Total number of cells per well}) \times (\text{Desired MOI}) = \text{Total transducing units needed (TU)}$$

$$(\text{Total TU needed}) \div (\text{TU/ml reported on C of A}) = \text{Total mL of lentiviral particles to add to each well}$$

Day 3

1. Remove the medium containing lentiviral particles from wells. Add fresh medium to a volume of 120 µL to each well.

Note: For cell types that do not strongly adhere to the plate, 100 µL of medium may be removed and replaced with 100 µL of fresh medium.

Day 4

1. Remove medium from wells. Add fresh medium containing puromycin.

Note: The appropriate concentration of puromycin for each cell type will vary. If the appropriate concentration for the desired cell type is unknown, a titration experiment, or kill curve, must be performed. Typically, 2–10 µg/mL are sufficient to kill most untransduced mammalian cell types.

Puromycin Titration:

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 medium.
2. The next day add 500–10,000 ng/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 medium 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 on

1. Replace medium with fresh puromycin containing medium every 3–4 days until resistant colonies can be identified.
2. Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.
3. A variety of phenotypic, enzymatic, or gene expression assays may be performed. Each assay should be optimized prior to the high-content screen with both negative and positive controls.

Note: Due to the random integration of the lentivirus into the host genome, varying levels of target gene knockdown may be seen with different puromycin resistant colonies. Testing a number of colonies will allow the optimal degree of knockdown to be determined.

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.
	MOI is too low.	Transduce at a higher MOI.
	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.
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.⁷

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 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 Non-Target 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 mouse genes. The short-hairpin sequence contains 5 base pair mismatches to any known 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 TurboGFP 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 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 mouse genes, it can also be used as a negative non-target control in shRNA experiments

Mouse Cell Type Table

The mouse cell types listed below have been successfully infected by pLKO.1-puro based shRNA constructs

Cell lines	Cell Type
NIH 3T3	fibroblast
Primary cells	Cell Type
astrocytes	normal
C3H10T1/2	mesenchymal
ECS	mouse embryonic stem cells
fibroblasts	mouse embryonic fibroblasts
MC3T3-E1	mouse bone marrow derived
molar mesenchymal	mouse embryonic mesenchymal

Reviews Indicating the Importance of Each of the Gene Family Sets:

Kinases

1. Gomase, V.S. et al., *Curr. Drug Metab.*, **9**, 255-8 (2008).

Phosphatases

1. Hendriks, W.J. et al., Protein tyrosine phosphatases: functional inferences from mouse models and Murine diseases. *FEBS J.*, **275**, 816-30 (2008).
2. Tremblay, M.L., and Giguère, V., Phosphatases at the heart of FoxO metabolic control. *Cell Metab.*, **7**, 101-3 (2008).
3. Heideker, J. et al., Phosphatases, DNA damage checkpoints and checkpoint deactivation. *Cell Cycle*, **6**, 3058-64 (2007).
4. Sawyer, T.K. et al., Protein phosphorylation and signal transduction modulation: chemistry perspectives for small-molecule drug discovery. *Med. Chem.*, **1**, 293-319 (2005).

Tyrosine Kinases

1. Annerén, C., Tyrosine kinase signaling in embryonic stem cells. *Clin Sci (Lond)*, **115**, 43-55 (2008).
2. Comoglio, P.M. et al., Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov*, **7**, 504-16 (2008).
3. Ren, H. et al., Receptor tyrosine kinases as therapeutic targets in malignant glioma cells. *Rev Recent Clin. Trials*, **2**, 87-101 (2007).

References

1. Demedts, I.K. et al., Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respiratory Research*, **7**, 53 (2006).
2. Zufferey, R. et al., Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* **15**, 871-85 (1997).
3. Zufferey, R. et al., Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J. Virol.*, **72**, 9873-80 (1998).
4. 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).
5. Stewart, S.A. et al., Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
6. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (<http://www4.od.nih.gov/oba>).
7. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).

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