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Product Information

MISSION® shRNA Human Gene Family Set
Transduction Particles

Catalog Numbers: **SH0131, SH0231, SH0431, SH0531, SH0731, SH0831, SH1031, SH1131, SH1331, SH1831, SH1931, SH2131, SH2231, SH2331, SH2431, SH2531, SH2631, SH2731, SH2831, SH2931, SH3031**

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. We have collected a list of reviews that highlight the importance of each gene family set.

The MISSION shRNA Transduction Particles Set allows for high throughput loss-of-function and genetic interaction screens. The 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 efficiencies, 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 (HEK293T) by co-transfection with compatible packaging plasmids.[5,6] 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.[7] 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,[4] overcoming low transfection and integration difficulties when using these cell lines.

Please see the **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 assay, 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/Reagents

Each individual construct is provided as 4 x 50 μL of frozen stock containing 10^6 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 into 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 search 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).

Materials Suggested but Not Provided

- Hexadimethrine bromide, Catalog Number H9268
- Puromycin Ready Made Solution (10 mg/ml in H_2O), 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

Precautions and Disclaimer

These products are 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.[9] 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.

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.

Preparation Instructions

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

Procedure

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

Day 1.

a. Add 1.6 x 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.
b. Incubate 18–20 hours at 37 °C in a humidified incubator in an atmosphere of 5–7% CO_2.

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.

a. Remove medium from wells. To each well add 110 μL 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.

b. 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% CO_2. 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 are 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 x 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 (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:
(Total number of cells per well) x (Desired MOI) = Total transducing units needed (TU)

(Total TU needed) / (TU/ml reported on C of A) = Total ml of lentiviral particles to add to each well

Day 3.
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 fresh medium.

Day 4.
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 x 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 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 on
a. Replace medium with fresh puromycin containing medium every 3–4 days until resistant colonies can be identified.
b. Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.
c. 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.

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Catalog Number	Gene Family Set	Gene Count*	Clone Count*	Average Number Clones/Gene*
SH1931	Apoptosis Pathway	440	2882	6.6
SH2931	B-Cell Activation	99	563	5.7
SH2231	Cell Adhesion Genes	366	2013	5.5
SH0831	Cytokine and Chemokine	106	525	5.0
SH1331	Cytokine and Chemokine Receptors	94	540	5.7
SH2331	Cytoskeleton Genes	273	1534	5.6
SH3031	Epigenetic Regulators	10	55	5.5
SH1831	DNA Repair Pathway	116	658	5.7
SH0731	Ubiquitin Hydrolases (DUBS)	124	612	4.9
SH2531	Extracellular Matrix Genes	330	1750	5.3
SH0231	G-Protein Coupled Receptors (GPCRs)	444	2761	6.2
SH2631	Helicase	133	676	5.1
SH1031	Ion Channel	276	1357	4.9
SH2731	JAK-STAT Pathway	189	1128	6.0
SH0131	Kinases, complete	513	5141	10.0
SH1131	Nuclear Hormone Receptors	46	218	4.7
SH2431	p53 Pathway	239	1502	6.3
SH0431	Phosphatases	299	2369	7.9
SH2831	T-Cell Activation	240	1265	5.3
SH0531	Tumor Supressors	73	443	6.1
SH2131	Ubiquitin Ligases (E1, E2, E3)	203	1025	5.0

*The MISSION Production and Bio-informatics Team constantly reviews and confirms the clones available for each 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 exact TRCN clone numbers and their plate location.

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

The recommended controls for any shRNA experiment are described in the **Control Selection Table** and are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[8]

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 No. 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 No. 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 TurboGFP™ Control Transduction Particles, Catalog No. 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 # 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

Cell lines, human	**Cell Type**	**Cell lines, human**	**Cell Type**	**Primary cells human**	**Cell Type**
HEK293	embryonic kidney cells	A431	epidermal carcinoma	dendritic	immature dendritic
HeLa	cervical adenocarcinoma	THP1	monocytic	T-cells	lymphocytes
A549	lung adenocarcinoma	RAW264.7	macrophage	epithelial	prostate
H1299	lung carcinoma	SH-SY5Y	brain neuroblastoma	fibroblasts	primary mammary
HT29-D4	colon carcinoma	HCN-1A	brain cortical neuron	**Primary cells, other species**	**Cell Type**
HepG2	hepatocellular carcinoma	SupT1	T-cells	ECS	mouse embryonic stem cells
HCT116	colon carcinoma	BJ-TERT	diploid fibroblasts	fibroblasts	mouse embryonic fibroblasts
MCF7	breast carcinoma	**Cell lines, mouse**	**Cell Type**	MC3T3-E1	mouse bone marrow derived
MCF10A	breast carcinoma	NIH3T3	fibroblast	molar mesenchymal	mouse embryonic mesenchymal
Panc-1	pancreatic epithelioid carcinoma	**Primary cells, human**	**Cell Type**	cardiomyocytes	rat neonatal cardiomyocytes
PC3	prostate carcinoma	astrocytes	normal		
DU145	prostate carcinoma	C3H10T1/2	mesenchymal		

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