

## Product Information

### MISSION® shRNA Human Gene Family Sets, Bacterial Glycerol Stocks

Catalog Numbers: **SH0111, SH0211, SH0411, SH0511, SH0711, SH0811, SH1011, SH1111, SH1311, SH1811, SH1911, SH2111, SH2211, SH2311, SH2411, SH2511, SH2611, SH2711, SH2811, SH2911, and SH3011**

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 Gene Family Sets allow for high throughput loss-of-function and genetic interaction screens. The glycerol stock format consists of bacterial glycerol stocks harboring sequence-verified shRNA lentiviral plasmid vectors. Each MISSION shRNA clone is constructed within the lentivirus plasmid vector pLKO.1-puro.<sup>1</sup> The pLKO.1-puro vector contains the ampicillin and puromycin antibiotic resistance genes for selection of inserts in bacterial or mammalian cells, respectively. The sets consist of sequence-verified shRNA lentiviral plasmid DNA. For each gene target, there are 3 or more constructs that have been designed against each target gene using a proprietary algorithm. Therefore, a range of gene silencing 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 range of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

Bacterial cultures may be amplified from the glycerol stocks for use in purification of the shRNA plasmid DNA. Subsequently, target cell lines may be transfected with the purified plasmid for transient or stable gene silencing (puromycin selection). In addition, self-inactivating replication incompetent viral particles can be produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.<sup>4-5</sup> 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,<sup>6</sup> 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.

Each MISSION shRNA clone is constructed within the lentiviral plasmid vector pLKO.1-puro<sup>6</sup> followed by transformation into *Escherichia coli*. The pLKO.1-puro vector contains bacterial (ampicillin) and mammalian (puromycin) antibiotic resistance genes for selection of inserts in either bacterial or mammalian cell lines.

### Components/Reagents

The individual clones are provided as a 50 µl bacterial glycerol stock containing Terrific Broth (TB), carbenicillin at 100 µg/ml, and 15% glycerol. The sets are provided in 96-well barcoded plates, along with a CD containing gene description, symbol, RefSeq, locus link, clone ID, hairpin sequence, and plate map position for each clone. The number of plates will vary between gene families; we will not break up a target set between plates.

The hairpin sequence and other unique clone information may be obtained by searching the MISSION search database at: [www.sigma.com/yfg](http://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.

#### Genotype of host *E. coli* strain

F<sup>-</sup>  $\Phi$ 80/*lacZ* $\Delta$ M15  $\Delta$ (*lacZYA-argF*)U169 *endA1 recA1 relA1 gyrA96 hsdR17* (*r<sub>k</sub><sup>-</sup>*, *m<sub>k</sub><sup>+</sup>*) *phoA supE44 thi-1 tonA*

#### 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.

#### Storage/Stability

Stable for at least six months after receipt when stored at -70 °C. Avoid repeated freeze/thaw cycles, which will severely reduce culture viability.

Catalog Number	Human Gene Family Set	Gene Count *	Clone Count *	Average Number Clones/Gene *
SH1911	Apoptosis Pathway	443	3512	7.9
SH2911	B-Cell Activation	99	661	6.7
SH2211	Cell Adhesion Genes	368	2396	6.5
SH0811	Cytokine and Chemokine	106	538	5.1
SH1311	Cytokine and Chemokine Receptors	93	584	6.3
SH2311	Cytoskeleton Genes	275	1991	7.2
SH3011	Epigenetic Regulators	10	59	5.9
SH1811	DNA Repair Pathway	117	837	7.2
SH0711	Ubiquitin Hydrolases (DUBS)	127	830	6.5
SH2511	Extracellular Matrix Genes	331	1968	5.9
SH0211	G-Protein Coupled Receptors (GPCRs)	541	2864	5.3
SH2611	Helicase	136	909	6.7
SH1011	Ion Channel	277	1479	5.3
SH2711	JAK-STAT Pathway	190	1358	7.1
SH0111	Kinases, complete	678	7607	11.2
SH1111	Nuclear Hormone Receptors	218	1448	6.6
SH2411	p53 Pathway	242	1865	7.7
SH0411	Phosphatases	320	2099	6.6
SH2811	T-Cell Activation	242	1469	6.1
SH0511	Tumor Suppressors	73	575	7.9
SH2111	Ubiquitin Ligases (E1, E2, E3)	349	2151	6.2

\*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.



### Troubleshooting Guide

Problem	Cause	Solution
No growth of bacterial culture on selection plates	Incorrect carbenicillin concentration	Re-check the carbenicillin concentration or pour fresh plates containing 100 µg/ml of carbenicillin.
	Insufficient inoculum volume from frozen culture	Remove a larger volume of culture from the frozen glycerol.
	Insufficient storage temperature of frozen culture	Storage temperature must be -70 °C or lower. Obtain new stock.
	Multiple freeze-thaw cycles	Avoid freeze thawing the culture more than 2 times.
Low plasmid yield	Difficult construct	Perform larger purifications (midi or maxi preps) on constructs that produce low yields.
	Failure to use a single colony for inoculation	Use an isolated colony for inoculation of cultures for DNA preps

### 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.<sup>7</sup>

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will provide a reference point for comparing all other samples.
Negative Control: Transfection with empty vector, containing no shRNA insert	MISSION pLKO.1-puro Control Vector, Catalog Number SHC001 The empty vector, pLKO.1-puro, is a useful negative control that will not activate the RNAi pathway because it does not contain an shRNA insert. It will allow for observation of cellular effects of the transfection process and the delivery of the lentiviral vector. Cells transfected with the empty vector provide a useful reference point for comparing specific knockdown.
Negative Control: Transfection with non-targeting shRNA	MISSION Non-Target shRNA Control Vector, Catalog Number SHC002 This non-targeting shRNA vector 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 transfection on gene expression. Cells transfected with the non-target shRNA vector will also provide a useful reference for interpretation of knockdown.
Positive Control: Transfection with positive reporter vector	MISSION TurboGFP™ Control Vector, Catalog Number SHC003 This vector is a useful positive control for measuring transfection efficiency and optimizing shRNA delivery. The TurboGFP Control Vector consists of the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Transfection of this vector provides fast visual confirmation of successful transfection and delivery.
Positive Control: Transfection with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Vector, Catalog Number SHC004 The TurboGFP shRNA vector consists of the pLKO.1-puro vector, containing shRNA that targets TurboGFP, and can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA Control Vector has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this vector 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	<b>Primary cells, other species</b>	<b>Cell Type</b>
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	<b>Cell lines, mouse</b>	<b>Cell Type</b>	MC3T3-E1	mouse bone marrow derived
MCF10A	breast carcinoma	NIH3T3	fibroblast	molar mesenchymal	mouse embryonic mesenchymal
Panc-1	pancreatic epithelioid carcinoma	<b>Primary cells, human</b>	<b>Cell Type</b>	cardiomyocytes	rat neonatal cardiomyocytes
PC3	prostate carcinoma	astrocytes	normal		
DU145	prostate carcinoma	C3H10T1/2	mesenchymal		

### Reviews Indicating the Importance of Each of the Gene Family Sets-

#### Apoptosis Pathway

1. Krysko, D.V. *et al.*, Apoptosis and necrosis: detection, discrimination and phagocytosis. *Methods*, **44**, 205-21 (2008).
2. Howley, B., and Fearnhead, H.O., Caspases as therapeutic targets. *J. Cell Mol. Med.*, Feb 24 [Epub ahead of print] (2008)
3. Logue, S.E., and Martin, S.J., Caspase activation cascades in apoptosis. *Biochem. Soc. Trans.* **36 (Pt 1)**, 1-9 (2008).

#### B Cell Activation

1. Tolar, P. *et al.*, Viewing the antigen-induced initiation of B-cell activation in living cells. *Immunol Rev.*, **221**, 64-76 (2008).
2. Youinou, P., B cell conducts the lymphocyte orchestra. *J. Autoimmun.*, **28**, 143-51. (2007).

#### Cell Adhesion

1. Ebnet, K., Organization of multiprotein complexes at cell-cell junctions. *Histochem. Cell Biol.*, Mar 26 [Epub ahead of print] (2008).
2. Basson, M.D., An intracellular signal pathway that regulates cancer cell adhesion in response to extracellular forces. *Cancer Res.*, **68**, 2-4 (2008).
3. Mousa, S.A., Cell adhesion molecules: potential therapeutic & diagnostic implications. *Mol. Biotechnol.*, **38**, 33-40. (2008).

#### Cytokine and Chemokine Receptors

1. Callewaere, C. *et al.*, Chemokines and chemokine receptors in the brain: implication in neuroendocrine regulation. *J. Mol. Endocrinol.*, **38**, 355-63 (2007)
2. Allen, S.J. *et al.*, Chemokine: receptor structure, interactions, and antagonism. *Annu. Rev. Immunol.*; **25**, 787-820 (2007).
3. Zlotnik, A. *et al.*, The chemokine and chemokine receptor superfamilies and their molecular evolution. *Genome Biol.*; **7**, 243 (2006).
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### Cytokines and Chemokines

1. Anderson, P. Post-transcriptional control of cytokine production. *Nat. Immunol.*, **9**, 353-9 (2008).
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### Cytoskeleton

1. Dalby, M.J., and Yarwood, S.J., Analysis of focal adhesions and cytoskeleton by custom microarray. *Methods Mol. Biol.*, **370**, 121-34 (2007).
2. Dustin, M.L., Cell adhesion molecules and actin cytoskeleton at immune synapses and kinapses. *Curr. Opin. Cell Biol.*, **19**, 529-33 (2007).

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2. Hakem, R., DNA-damage repair; the good, the bad, and the ugly. *EMBO J.*, **27**, 589-605 (2008).
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### DUBS - Ubiquitin Hydrolyases

1. Nicholson, B. *et al.*, Deubiquitinating enzymes as novel anticancer targets. *Future Oncol.*, **3**, 191-9 (2007).
2. Millard, S.M., and Wood, S.A., Riding the DUBway: regulation of protein trafficking by deubiquitylating enzymes. *J. Cell Biol.*, **173**, 463-8 (2006).
3. Amerik, A.Y., and Hochstrasser, M., Mechanism and function of deubiquitinating enzymes. *Biochim. Biophys. Acta*, **1695**, 189-207 (2004).

### Epigenetic Regulators

1. Esteller, M., Epigenetics in cancer. *N. Engl. J. Med.*, **358**, 1148-59. Review (2008).
2. Grønbaek, K. *et al.*, Epigenetic changes in cancer. *APMIS*, **115**, 1039-59 (2007).

### Extracellular Matrix

1. Rees, M.D. *et al.*, Oxidative damage to extracellular matrix and its role in human pathologies. *Free Radic. Biol. Med.*, Apr 8 (2008). [Epub ahead of print]
2. Adair-Kirk, T.L., and Senior, R.M., Fragments of extracellular matrix as mediators of inflammation. *Int. J. Biochem. Cell Biol.*, **40**, 1101-10 (2008).
3. Daley, W.P. *et al.*, Extracellular matrix dynamics in development and regenerative medicine. *J. Cell Sci.*, **121(Pt 3)**, 255-64 (2008).

### G-Protein-Coupled Receptors:

1. Thompson, M.D. *et al.*, G protein-coupled receptors disrupted in human genetic disease. *Methods Mol. Biol.*; **448**, 109-37 (2008).
2. Milligan, G., New aspects of G-protein-coupled receptor signalling and regulation. *Trends Endocrinol. Metab.*, **9**, 13-9 (1998).

### Helicases

1. Ha, T., Need for speed: mechanical regulation of a replicative helicase. *Cell*, **129**, 1249-50 (2007).
2. Singleton, M.R. *et al.*, Structure and mechanism of helicases and nucleic acid translocases. *Annu. Rev. Biochem.*, **76**, 23-50 (2007).
3. Xi, X.G., Helicases as antiviral and anticancer drug targets. *Curr. Med. Chem.*, **14**, 883-915 (2007).

### Ion Channels

1. Cannon, S.C., Physiologic principles underlying ion channelopathies. *Neurotherapeutics*, **4**, 174-83 (2007).

### JAK-STAT Pathway

1. Murray, P.J., The JAK-STAT signaling pathway: input and output integration. *J. Immunol.*, **178**, 2623-9 (2007).
2. O'Sullivan, L.A. *et al.*, Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol. Immunol.*, **44**, 2497-506 (2007).

### Kinases

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

### Nuclear Hormone Receptors

1. Kininis, M., and Kraus, W.L., A global view of transcriptional regulation by nuclear receptors: gene expression, factor localization, and DNA sequence analysis. *Nucl. Recept. Signal*, **6**, e005 (2008).

### p53 Pathway

1. Bose, I., and Ghosh, B., The p53-MDM2 network: from oscillations to apoptosis. *J. Biosci.*, **32**, 991-7 (2007).
2. Efeyan, A., and Serrano, M., p53: guardian of the genome and policeman of the oncogenes. *Cell Cycle*, **6**, 1006-10 (2007).
3. Kastan, M.B., Wild-type p53: tumors can't stand it. *Cell*, **128**, 837-40 (2007).

## Phosphatases

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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).

## T Cell Activation

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2. Brenner, D. *et al.*, Concepts of activated T cell death. *Crit. Rev. Oncol. Hematol.*, **66**, 52-64 (2008).
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4. Lämmermann, T, and Sixt, M., The microanatomy of T-cell responses. *Immunol. Rev.*, **221**, 26-43 (2008).

## Tumor Suppressors

1. Vattemi, E., and Claudio, P.P., Tumor suppressor genes as cancer therapeutics. *Drug News Perspect*, **20**, 511-20 (2007).
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## Ubiquitin Ligases (E1, E2, E3)

1. Cardozo, T., and Pagano, M., Wrenches in the works: drug discovery targeting the SCF ubiquitin ligase and APC/C complexes. *BMC Biochem.*, **8 Suppl 1**, S9 (2007).
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3. Sun, Y., Overview of approaches for screening for ubiquitin ligase inhibitors. *Methods Enzymol.*, **399**, 654-63 (2005).
4. Hershko, A., The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. *Cell Death Differ.*, **12**, 1191-7 (2005).

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