

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.¹ 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.⁴⁻⁵ 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,⁶ 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-puro⁶ 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 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⁻ Φ 80/*lacZ* Δ M15 Δ (*lacZYA-argF*)U169 *endA1 recA1 relA1 gyrA96 hsdR17* (*r_k⁻, m_k⁺*) *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.⁷

| 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 | 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 | | |

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)
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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).

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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).
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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).
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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).
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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).
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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

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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).

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3. Heideker, J. *et al.*, Phosphatases, DNA damage checkpoints and checkpoint deactivation. *Cell Cycle*, **6**, 3058-64 (2007).
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1. Won, J., and Lee, G.H., T-cell-targeted signaling inhibitors. *Int. Rev. Immunol.*, **27**, 19-41 (2008).
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