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Product Information

MISSION® shRNA Human Gene Family Sets , DNA Format

Catalog Numbers **SH0121, SH0221, SH0421, SH0521, SH0721, SH0821, SH1021, SH1121, SH1321, SH1821, SH1921, SH2121, SH2221, SH2321, SH2421, SH2521, SH2621, SH2721, SH2821, SH2921, SH3021**
Storage Temperature –20 °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 included a table of reviews for each gene family set.

The MISSION shRNA Human Gene Family Sets, DNA Format, contains shRNA clones that allows for high throughput loss-of-function and genetic interaction screens. 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 set consists of sequence-verified shRNA lentiviral plasmid DNA. Each target set consists of 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 series of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

RNAi knockdown be achieved either with the plasmid DNA or the MISSION lentiviral delivery system. 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.[2-3] Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based particles permit efficient infection and genomic integration of the specific shRNA construct into differentiated and non-dividing cells, such as neurons and dendritic cells, 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.

Components/Reagents

The individual constructs are provided as 40 µL frozen stocks containing an average of 2 µg of plasmid DNA in Tris-EDTA (TE) buffer with amounts of DNA ranging from 400 ng to 4 µg per well.
Sets are provided in 96-well plates with a one dimensional barcode label on each plate and a CD containing plate map positions.

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.

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

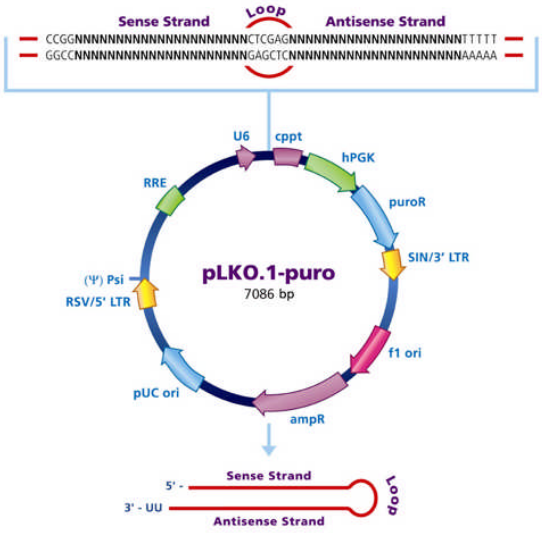
All components are stable for at least one year after receipt when stored at –20 °C.

Catalog Number	Gene Family Set	Gene Count *	Clone Count *	Average Number Clones/Gene *
SH1921	Apoptosis Pathway	440	2882	6.6
SH2921	B-Cell Activation	99	563	5.7
SH2221	Cell Adhesion Genes	366	2013	5.5
SH0821	Cytokine and Chemokine	106	525	5.0
SH1321	Cytokine and Chemokine Receptors	94	540	5.7
SH2321	Cytoskeleton Genes	273	1534	5.6
SH3021	Epigenetic Regulators	10	55	5.5
SH1821	DNA Repair Pathway	116	658	5.7
SH0721	Ubiquitin Hydrolases (DUBS)	124	612	4.9
SH2521	Extracellular Matrix Genes	330	1750	5.3
SH0221	G-Protein Coupled Receptors (GPCRs)	444	2761	6.2
SH2621	Helicase	133	676	5.1
SH1021	Ion Channel	276	1357	4.9
SH2721	JAK-STAT Pathway	189	1128	6.0
SH0121	Kinases, complete	513	5141	10.0
SH1121	Nuclear Hormone Receptors	46	218	4.7
SH2421	p53 Pathway	239	1502	6.3
SH0421	Phosphatases	299	2369	7.9
SH2821	T-Cell Activation	240	1265	5.3
SH0521	Tumor Supressors	73	443	6.1
SH2121	Ubiquitin Ligases (E1, E2, E3)	203	1025	5.0

*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.

Lentiviral Plasmid Vector pLKO.1-puro Features

Name	Description
U6	U6 Promoter
cppt	Central polypurine tract
hPGK	Human phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
SIN/3′ LTR	3' self inactivating long terminal repeat
f1 ori	f1 origin of replication
ampR	Ampicillin resistance gene for bacterial selection
pUC ori	pUC origin of replication
5' LTR	5' long terminal repeat
Psi	RNA packaging signal
RRE	Rev response element



Control Selection Table

Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[4]

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			

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