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Product Information

MISSION® shRNA Bacterial Glycerol Stock

Catalog Number **SHCLNG**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Small interfering RNAs (siRNAs) processed from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) in mammalian cells. The MISSION product line is a viral-vector-based RNAi library against annotated mouse and human genes. shRNAs that are processed into siRNAs intracellularly are expressed from 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.

The TRC1 and TRC1.5 libraries consist of bacterial glycerol stocks harboring sequence-verified shRNA lentiviral plasmid vectors for mouse and human genes cloned into the pLKO.1-puro vector (see Figure 1). The TRC2 library consists of bacterial glycerol stocks harboring sequence-verified shRNAs for mouse and human genes in the TRC2-pLKO-puro vector (see Figure 2). The TRC2 vector has a single additional element in comparison to the TRC1 vector. This is the WPRE,[1] or the Woodchuck Hepatitis Post-Transcriptional Regulatory Element. WPRE allows for enhanced expression of transgenes delivered by lentiviral vectors.[2]

A number of individual shRNAs designed using a proprietary algorithm are available for each gene. We recommend purchasing multiple individual constructs (the recommended number is listed on each clone ordering page) targeting different regions of the gene sequence.

A range of knockdown efficiencies can be expected when using multiple 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.

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.[3-4] 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.

Each MISSION shRNA clone is constructed within the lentivirus plasmid vector, pLKO.1-puro[5] or TRC2-pLKO-puro, followed by transformation into *Escherichia coli*. Both vectors contain 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 frozen bacterial glycerol stock containing Terrific Broth (TB), carbenicillin at 100 μg/ml and 15% glycerol.

Orders of 25 or fewer clones are provided in 1.4 ml TrakMates® tubes. Each TrakMates tube contains a unique 2 dimensional barcode on the bottom of the tube that can be read using a corresponding reader. A printed value corresponding to The RNAi Consortium (TRC) clone number is also provided on each tube. Orders of >25 clones are provided in a 96-well plate with a one dimensional barcode label on the plate. 96-well plates are provided with a CD containing plate map positions.

The hairpin sequence, other unique clone information and additional gene related products including antibodies and small molecules can be found through our comprehensive search tool, Your Favorite Gene www.sigma-aldrich.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$^-$ Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17 (r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *ton*A

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 guaranteed to be stable for at least six months after receipt when stored at –70 °C. Repeated freeze/thaw cycles are to be avoided.

Procedure
Culturing Clonal Cell Lines

Gently spin down material in tubes before opening.

1. Remove ice splinters (50-100 µL) from the frozen bacterial glycerol stock using a sterile loop and place them into a sterile culture tube containing 0.5 ml TB **without** antibiotics.
2. Incubate the culture at 37 °C with shaking for 15–30 minutes.
3. Using a sterile loop, streak 25-50 µL of the incubated culture onto freshly prepared plates containing LB agar and carbenicillin, Catalog Number C3416, 100 µg/ml. Carbenicillin, an ampicillin analog, is recommended over ampicillin due to its increased stability in cultures.
4. Incubate plates in a humidified atmosphere for 15–20 hours at 37 °C.
5. Isolate a single colony from the plate and use as a source inoculum for downstream applications (e.g., plasmid DNA preparation).

Figure 1. TRC1 and TRC1.5 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

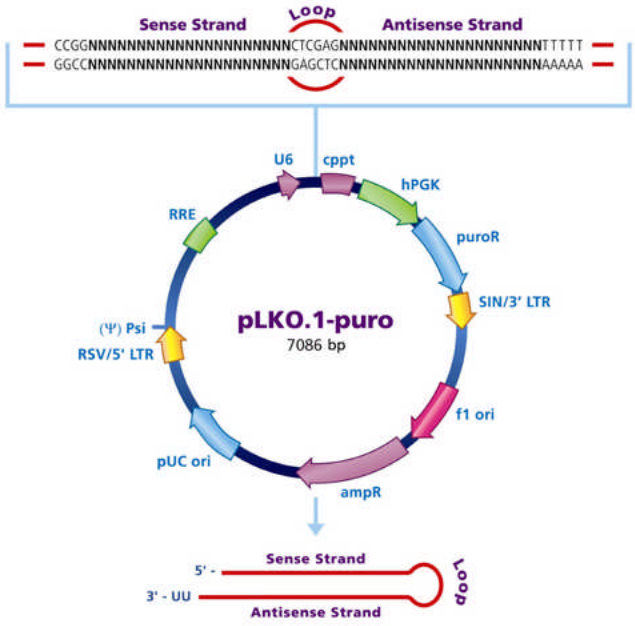
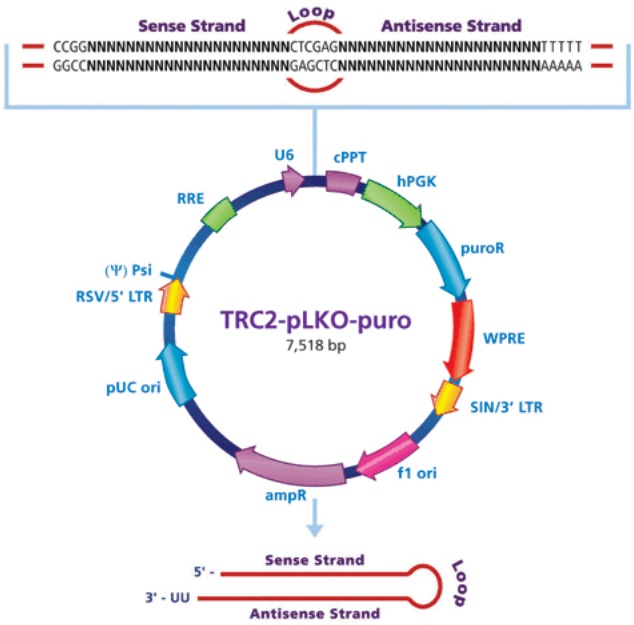


Figure 2. TRC2 Lentiviral Plasmid Vector TRC2-pLKO-puro Features

Name	Description
U6	U6 Promoter
cppt	Central polypurine tract
hPGK	Human phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
WPRE	Woodchuck Hepatitis Post-Transcriptional Regulatory Element
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



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.
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

Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[6] Additional controls are available. Please visit:
http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/trc-shrna-products/shrna-controls.html

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 Control Vector Purified DNA TurboGFP™, 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 HEK293T 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. Optimal conditions will need to be determined for your experimental needs. For the most updated cell line list, and some guidelines for conditions, please visit:
http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/getting-started.html

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			

References

1. Donello, J.E., *et al.*, Woodchuck hepatitis virus contains a tripartite posttranscriptional regulatory element. *J Virol*., **72**, 5085-5092 (1998).
2. Zufferey, R., *et al.*, Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J Virol*., **73**, 2886-2892 (1999).
3. Zufferey, R., *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* **15**, 871-885 (1997).
4. Zufferey, R., *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J Virol*., **72**, 9873-9880 (1998).
5. Stewart, S.A., *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
6. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).

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