3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

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 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

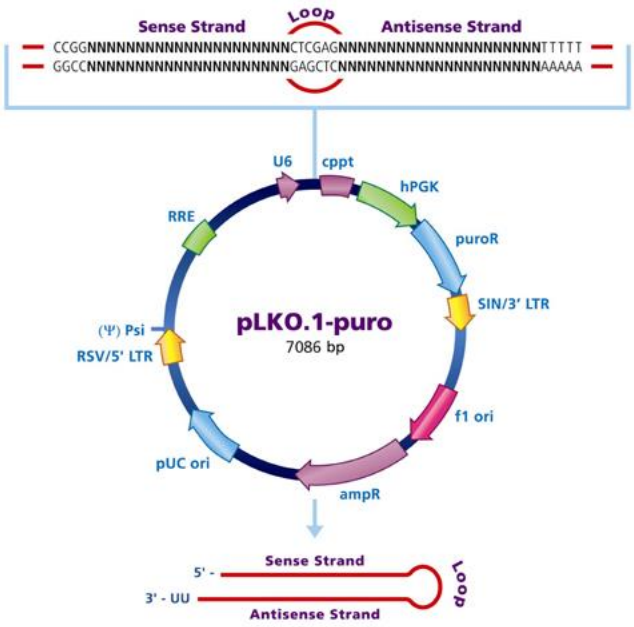
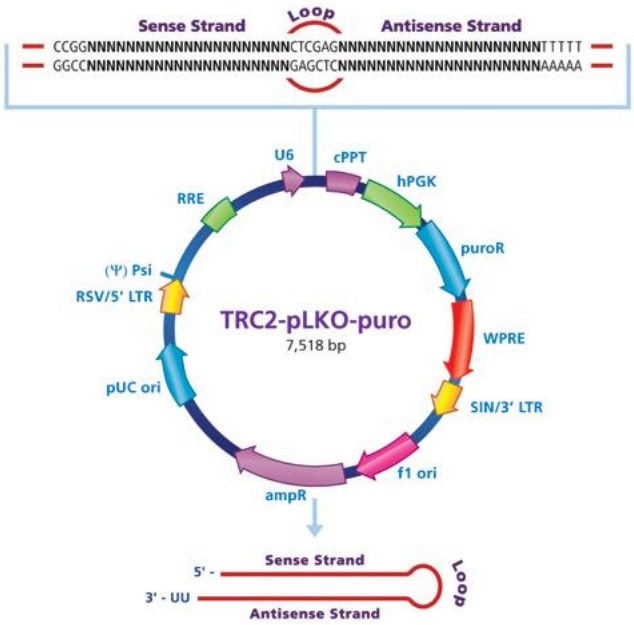


Figure 2. TRC2 Lentiviral Plasmid Vector TRC2-pLKO-puro Features

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).

MISSION is a registered trademark of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.
TrakMates is a registered trademark of Matrix Technologies Corporation.
TurboGFP is a trademark of Evrogen Co.

Limited Use Licenses
Use of this product for Commercial Purposes requires a license from Sigma-Aldrich Corporation. The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration, but excludes not-for-profit core facilities providing services within their own research institutions at cost. Core facilities are invited to join Sigma-Aldrich's RNAi Partnership Program. Details of Sigma-Aldrich's RNAi Partnership Program can be found at **www.sigma.com/rpp**.

The MISSION shRNA Library of The RNAi Consortium is produced and distributed under license from the Massachusetts Institute of Technology.

Licensed under Carnegie Institution US Patent 6,506,559 and Massachusetts Institute of Technology and for laboratory and commercial research use only.

This product is licensed under U.S. Pat. Nos. 5,817,491; 5,591,624; 5,716,832; 6,312,682; 6,669,936; 6,235,522; 6,924,123 and foreign equivalents from Oxford BioMedica (UK) Ltd., Oxford, UK, and is provided for use in academic and commercial *in vitro* and *in vivo* research for elucidating gene function, and for validating potential gene products and pathways for drug discovery and development, but excludes any use of LentiVector® technology for: creating transgenic birds for the purpose of producing useful or valuable proteins in the eggs of such transgenic birds, the delivery of gene therapies, and for commercial production of therapeutic, diagnostic or other commercial products not intended for research use where such products do not consist of or incorporate a lentiviral vector. Information about licenses for commercial uses excluded under this license is available from Oxford BioMedica (UK), Ltd., Medawar Center, Oxford Science Park, Oxford OX4 4GA UK **enquiries@oxfordbiomedica.co.uk** or BioMedica Inc., 11622 El Camino Real #100, San Diego CA 92130-2049 USA. LentiVector is a registered US and European Community trademark of Oxford BioMedica plc.

This product is licensed under agreement with Benitec Australia Ltd. and CSIRO as co-owners of U.S. Pat. No. 6,573,099 and foreign counterparts, for use in research to understand, diagnose, monitor, treat and prevent human diseases and disorders, including the use of animals for such research use, except that use of ddRNAi as a therapeutic agent or as a method of disease treatment, prevention, diagnosis or for disease monitoring is excluded. Information regarding licenses to these patents for use of ddRNAi as a therapeutic agent or for other uses excluded under this license is available from Benitec at **licensing@benitec.com**. Information about licenses for the use of ddRNAi in other fields, is available from CSIRO at **pi.csiro.au/RNAi**.

This product (based upon the lentikat system) is sub-licensed from Invitrogen Corporation under U.S. Patent Nos. 5,686,279, 5,834,256, 5,858,740; 5,994,136; 6,013,516; 6,051,427, 6,165,782, and 6,218,187 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for nonhuman research use requires a license from Cell Genesys, Inc. Please contact Cell Genesys, Inc. at 342 Lakeside Drive, Foster City, California 94404. Use of this technology to make or sell products or offer services for consideration in the research market requires a license from Invitrogen Corporation, 1600 Faraday Ave., Carlsbad, CA 92008.

This product is for non-clinical research use only. It is not to be used for commercial purposes. Use of this product to produce products for sale or for diagnostic, therapeutic or high throughput drug discovery purposes (the screening of more than 10,000 compounds per day) is prohibited. This product is sold under license from Invitrogen Corporation. In order to obtain a license to use this product for these commercial purposes, contact The Regents of the University of California. This product or the use of this product is covered by U.S. Patent No. 5,624,803 owned by The Regents of the University of California.

All Mission TRC II Lentiviral backbone-containing products contain a specific genetic component (WPRE), which is licensed from the Salk Institute for Biological Studies and covered under the following patents:
U.S. Patent No. 6,136,597 , U.S. Patent No. 6,284,469, U.S. Patent No. 6,312,912, U.S. Patent No. 6,287,814.

Purchaser Notification:
Licensee has a license to sell the Product containing WPRE, under the terms described below. Any use of WPRE outside of Licensee's Product or the Product's intended use, requires a license as detailed below. Before using the Product containing WPRE, please read the following license agreement. If you do not agree to be bound by its terms, contact Licensee within 10 days for authorization to return the unused Product containing WPRE and to receive a full credit.

Licensee grants you a non-exclusive license to use the enclosed Product containing WPRE in its entirety for its intended research use. The Product containing WPRE is being transferred to you in furtherance of, and reliance on, such license. Any use of WPRE outside of Licensee's Product or the Product's intended use including for Commercial Purposes, requires a license from the Salk Institute for Biological Studies. Commercial Purposes means any activity by a party for consideration, but excludes not-for-profit core facilities providing services within their own research institutions at cost. This license agreement is effective until terminated. You may terminate it at any time by destroying all Products containing WPRE in your control. It will also terminate automatically if you fail to comply with the terms and conditions of the license agreement. You shall, upon termination of the license agreement, destroy all Products containing WPRE in your control, and so notify Licensee in writing.

This License shall be governed in its interpretation and enforcement by the laws of the State of California.

Contact for WPRE Licensing:
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037
Attn.: Office of Technology Management
Phone: (858) 453-4100 extension 1703
Fax: (858) 546-8093

This Product is covered by US and foreign patent applications or patents and other proprietary intellectual property rights owned by CSHL ("CSHL shRNA IP Rights"), including U.S. Patent Nos. 8,153,776, 8,202,846, 8,383,599, 8,829,264, and EP1546174.

Subject to acceptance and all terms and conditions of this License, sale of the Product to Buyer by Sigma-Aldrich, Co. (acting under its license, an "Authorized Sale") conveys to Buyer only the nonexclusive, nontransferable right (with no right to sublicense) under the shRNA IP Rights to use the Product solely for Customer's internal research purposes, and only at its facility where the Product is delivered by Sigma-Aldrich, Co.

The Product is for research use only and may not be used *in vitro* or *in vivo* for any diagnostic, preventative, therapeutic or vaccine application, or used (directly or indirectly) in humans for any purpose.

Non-Profit Buyers. If Buyer is a Non-Profit Entity, then the following additional restrictions will apply:

Customer obtains no right to use, develop or otherwise exploit the product for any commercial purpose.

Commercial Buyers. If Buyer is a Commercial Entity, then the following additional restrictions will apply:

A Product sale is an Authorized Sale only if Buyer has already entered into a separate written agreement that has been executed by CSHL or Hairpin Technologies, that covers the CSHL shRNA IP Rights, and that is then currently in effect. Any delivery or transfer of Product to Customer outside of an Authorized Sale is void, conveys no implied or express right under this license and Customer will immediately return Product to Sigma-Aldrich for a refund.

"Commercial Entity" means any entity or organization other than a Non-Profit Entity.

"CSHL" means Cold Spring Harbor Laboratory.

"Hairpin Technologies" means Hairpin Technologies, Inc. located at 2200 Smithtown Avenue, Ronkonkoma, NY 11779, www.hairpintechnologies.com.

"Non-Profit Entity" means any college, university or governmental entity (including without limitation, governmental and quasi-governmental institutes and research laboratories), or any non-profit scientific, research or educational organization of the type described in section 501(c)(3) of the Internal Revenue Code or qualified under a state non-profit organization statute.

"Product" means a product (including, without limitation, expression vectors encoding a shRNA, the design, manufacture or use of which (in whole or in part) is the subject of the shRNA IP Rights, and is deemed to include all components, progeny, reproductions, modified versions and other derivatives thereof.

This license is subject to a license from CSHL or Hairpin Technologies, and CSHL and Hairpin Technologies reserves all other rights under its license. For information on licensing rights for Commercial Entities, including use of this product for purposes other than research and trial licenses, please contact Hairpin Technologies, Inc. at info@hairpintechnologies.com or call (631) 881-0844.

KEK,PHC,MAM 08/17-1

©2017 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.