SIGMA-ALDRICH®

sigma-aldrich.com

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® Lentiviral Transduction Particles

Catalog Number **SHCLNV**
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. MISSION shRNA clones permit rapid, cost-efficient loss-of-function and genetic interaction screens.

The Lentiviral Transduction Particles are produced from a library of sequence-verified lentiviral plasmid vectors for mouse and human genes. The TRC1 and TRC1.5 libraries consist of sequence-verified shRNAs cloned into the pLKO.1-puro vector (see Figure 1). The TRC2 library consists of sequence-verified shRNAs 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.

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,[3] overcoming low transfection and integration difficulties when using these cell lines. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.[4-5]

In addition, the lentiviral transduction particles are pseudotyped with an envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G), allowing transduction of a wide variety of mammalian cells including primary and embryonic stem cells.[6] The lentiviral transduction particles are titered via a p24 antigen ELISA assay and pg/ml of p24 are then converted to transducing units per ml using a conversion factor.

Components/Reagents

The individual constructs are provided in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin. There are several available options for volume, titer, and vector backbones.

Volumes available:

- 0.1 mL
- 0.2 mL
- 1.0 mL
- 2.0 mL
- 5.0 mL
- 10.0 mL

Titers available:

- 10^6 TU
- 10^7 TU
- 10^8 TU
- 10^9 TU

Note: not all volume and titer combinations available

	10^6 TU	10^7 TU	10^8 TU	10^9 TU
0.1 mL	NA	x	x	x
0.2 mL	x	x	x	x
1.0 mL	x	x	x	x
2.0 mL	x	x	x	x
5.0 mL	x	x	x	NA
10.0 mL	x	x	x	NA

Vector backbones available:
pLKO.1 (refers to the TRC version listed for the particular clone)
pLKO.1-CMV-Neo
pLKO.1-hPGK-Neo
pLKO.1-CMV-tGFP
pLKO.1-hPGK-Neo-CMV-tGFP
pLKO.1-hPGK-Puro-CMV-tGFP
Note: The customizable vectors are are based on the TRC1 vector.

Orders of 25 or fewer clones are provided in individual vials. Each vial contains a unique one dimensional barcode label 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.

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.

Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.[7] Follow all published RGL-2 guidelines for handling and waste decontamination. Also, use extra caution when using lentiviral transduction particles that express shRNA-targeting genes involved in cell cycle control, e.g., tumor suppressor genes.

Storage/Stability
All components are stable for at least six months after receipt when stored at –70 °C. Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer.

Related Products
- Hexadimethrine Bromide, Catalog Number H9268
- Puromycin dihydrochloride, Ready Made Solution, 10 mg/ml in H_2O, Catalog Number P9620
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- MISSION ExpressMag® 96-Well Magnetic Kit, Catalog Number SHM02
- PCR Reagents, please visit http://www.sigma-aldrich.com/pcr
- Prestige Antibodies, please visit http://www.sigma-aldrich.com/prestige
- Viability Assays

Procedure for the Use of MISSION Lentiviral Transduction Particles

Day 1
Plate the mammalian cell line of choice in complete medium 24 hours prior to transduction. Take into account the length of time that the cells will be cultured prior to performing RNAi analysis when determining plating density. Typically cells are transduced at 50-80% confluency.

Day 2
Thaw the lentiviral stock slowly on ice. Gently spin down material in tubes before opening. Add hexadimethrine bromide (the chemical equivalent of Polybrene) to the cells at a final concentration of 8 μg/ml.

Note: Hexadimethrine bromide enhances transduction of most cell types. However, some cells, such as primary neurons, are sensitive to hexadimethrine bromide. When using sensitive cells, do not add the hexadimethrine bromide and the cells should still be transduced.

Following addition of hexadimethrine bromide, gently swirl the plate to mix. Add the appropriate amount of viral particles at a suitable multiplicity of infection (MOI) and swirl the plate gently to mix. Incubate the cell-viral particle mixture at 37 °C overnight.

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.

To calculate MOI:
(total number of cells per well) x (desired MOI) = total transducing units needed (TU)

(total TU needed) / (TU/ml reported on C of A) = total ml of lentiviral particles to add to each well

Notes

a. When transducing a lentiviral construct into a cell line for the first time, it is recommended that a range of MOIs (0.5 – 20) be used to find the optimal degree of target knockdown.
b. When overnight incubation presents a toxicity concern, cells may be incubated for as little as 4 hours before changing the medium.

Day 3

Remove the viral particle-containing medium and replace it with fresh, pre-warmed complete culture medium.

Day 4

Perform one of the following based on whether the transduction experiment is transient or stable:

a. For transient expression experiments - Harvest the cells and assay for interference of the target gene. This can be done by a variety of methods such as qRT-PCR or Western blot.
b. For stable expression experiments - Remove the medium and replace it with fresh, complete medium that contains the appropriate amount of puromycin for selection of transduced cells. Proceed to day 5.

Note: When the appropriate concentration of puromycin for a specific cell type is unknown, perform a kill curve experiment. Typically, puromycin concentrations ranging from 0.5 – 10 μg/ml are sufficient to kill most untransduced mammalian cell lines.

Puromycin titration (kill curve) should be performed when working with a new cell type.

1. Plate 1.6 x 10^4 cells into wells of a 96-well plate with 120 μL fresh media.
2. The next day add 0.5 – 10 μg/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3 – 14 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the media containing puromycin every 3 days. The minimum concentration of puromycin that causes complete cell death after the desired time should be used for that cell type and experiment.

Note: Excess puromycin can cause many undesired phenotypic responses in most cell types.

Day 5 and forward

Replace medium with fresh, puromycin-containing medium every 3 – 4 days until resistant colonies can be identified (generally, 10 – 12 days after selection). Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.

Note: Due to the random integration of the lentivirus into the genome, varying levels of target gene knockdown may be seen from different puromycin-resistant clones. Testing a number of puromycin-resistant clones will allow a determination of which one provides the optimal degree of gene knockdown.

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

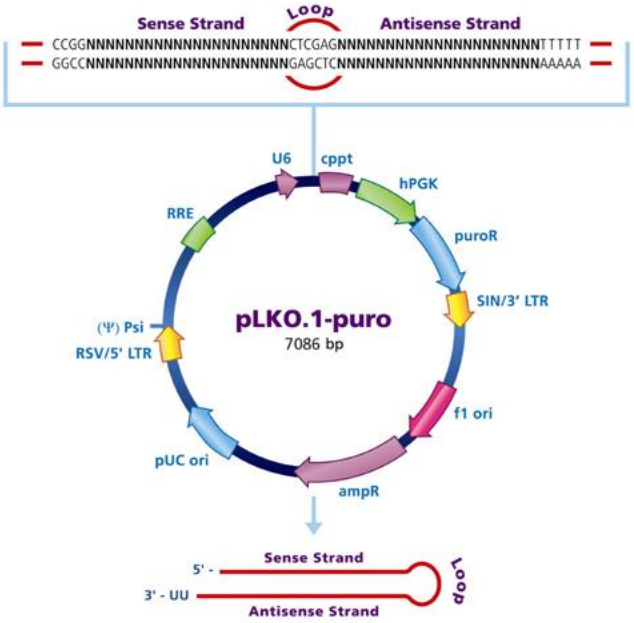
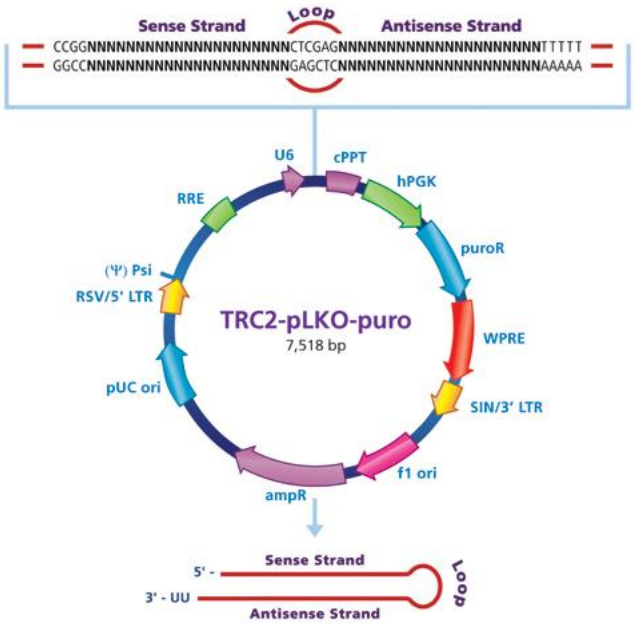


Figure 2. TRC2 Lentiviral Plasmid Vector TRC2-pLKO-puro Features

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
Low levels of target gene knockdown due to low transduction efficiency	Hexadimethrine bromide not included during transduction	Transduce in the presence of hexadimethrine bromide.
	Non-dividing cell type used	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag.
	MOI is too low	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag.
	Cells were harvested and assayed too soon after transduction	The shRNA must be permitted to accumulate in cells. Harvest 48-72 hours after transduction. Alternatively, knockdown results may be improved by placing cells under puromycin selection because untransduced cells will be killed.
No gene knockdown is observed	Viral stock stored incorrectly	Store stocks at −70 °C. Do not freeze/thaw more than 3 times.
	MOI is too low	Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as ExpressMag.
Cytotoxic effects observed after transduction	Target gene is essential for cell viability	Be sure that target gene is not essential for cell growth or viability.
	Hexadimethrine bromide was used during transduction	Be sure that cells are not sensitive to hexadimethrine bromide. Omit the hexadimethrine bromide during the transduction.
	Too much puromycin was used for selection	Determine the puromycin sensitivity of the cells by performing a kill curve and use the minimum concentration required to kill the untransduced cells.

Control Selection Table

Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[8] Additional controls are available. For a complete list, 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: Transduction with empty viral particles, containing no shRNA insert	MISSION pLKO.1-puro Control Transduction Particles, Catalog Number SHC001V The empty viral particles, produced from pLKO.1-puro, are a useful negative control that will not activate the RNAi pathway because they do not contain an shRNA insert. It will allow for observation of cellular effects of the transduction process. Cells transduced with the empty viral particles provide a useful reference point for comparing specific knockdown.
Negative Control: Transduction with non-targeting shRNA	MISSION Non-Target shRNA Control Transduction Particles, Catalog Number SHC002V This non-targeting shRNA 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 transduction on gene expression. Cells infected with the non-target shRNA will also provide a useful reference for interpretation of knockdown.
Positive Control: Transduction with positive reporter viral particles	MISSION TurboGFP™ Control Transduction Particles, Catalog Number SHC003V This is a useful positive control for measuring transduction efficiency and optimizing shRNA delivery. The TurboGFP Control transduction particles are produced from the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Transfection of this control provides fast visual confirmation of successful transduction.
Positive Control: Transduction with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Transduction Particles, Catalog Number SHC004V The TurboGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–puro vector containing shRNA that targets TurboGFP (Catalog # SHC004). These particles can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this shRNA 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. Stewart, S.A., *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, 9, 493-501 (2003).
4. Zufferey, R., *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* 15, 871-885 (1997).
5. Zufferey, R., *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J Virol.*, 72, 9873-9880 (1998).
6. Burns, J.C., *et al.*, Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors: Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA*, 90, 8033-8037 (1993).
7. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (http://www4.od.nih.gov/oba).
8. Whither RNAi? *Nature Cell Biology*, 5, 489-490 (2003).

MISSION and ExpressMag are registered trademarks of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.
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**.

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 (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