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Product Information

MISSION® shRNA Control Transduction Particles

Catalog Numbers **SHC001V, SHC002V, SHC003V, SHC004V, SHC005V, SHC007V, SHC008V, SHC009V, SHC010V, SHC011V, SHC012V, SHC013V, SHC014V, SHC015V, SHC016V, SHC201V, SHC202V, SHC203V, SHC204V, SHC216V, SHC312V, SHC314V, SHC317V, SHC332V, SHC334V, SHC337V, SHC001H, SHC002H, SHC003H, SHC004H, and SHC016H**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description
RNA interference (RNAi) is a powerful gene-specific silencing mechanism 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 types. In these cells, MISSION shRNA clones permit rapid, cost efficient loss-of-function and genetic interaction screens.

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,[1] overcoming low transfection and integration difficulties associated with these cell lines. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.[2,3] 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.[4]

Figure 1 depicts the base vector for all TRC1 and TRC1.5 clones (pLKO.1-puro). Figure 2 depicts the base vector for all TRC2 clones (TRC2-pLKO-puro). The TRC2 vector has a single additional element in comparison to the TRC1 vector. This element is the WPRE,[5] or the Woodchuck Hepatitis Post-Transcriptional Regulatory Element. WPRE allows for enhanced expression of transgenes delivered by lentiviral vectors.[6]

When conducting experiments using MISSION shRNA lentiviral particles, proper controls are a key element of experimental design to permit accurate interpretation of knockdown results and provide assurance of the specificity of the response observed. The MISSION shRNA Control Transduction Particles are lentiviral transduction particles that are useful as both positive and negative controls in experiments using the MISSION shRNA library.

Sigma's recommended controls for any shRNA experiment are provided in the **Control Selection Table** and are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[7] Please consult the Control Selection Table to select the controls that are most appropriate for your shRNA experiments. The **Quick Reference Guide** provides relevant insert sequence and gene target information specific to each product.

TRC1/TRC1.5 Controls
The TRC1 and TRC1.5 pLKO.1-puro Empty Vector Control Transduction Particles (SHC001V and SHC001H) do not contain a hairpin insert and provide a useful negative control that will not activate the RNA-induced silencing complex or RISC.

The TRC1 and TRC1.5 pLKO.1-puro Non-Mammalian shRNA Control Transduction Particles (SHC002V and SHC002H) are negative controls containing a sequence that should not target any known mammalian genes, but will engage with RISC. This control may cause some knockdown of tGFP, which should be taken into consideration when working with tGFP expressing cell lines.

The TRC1 and TRC1.5 pLKO.1-puro Non-Target shRNA Control Transduction Particles (SHC016V and SHC016H) target no known genes from any species. These non-mammalian and non-target controls serve as useful references for interpretation of knockdown results.

The TRC1 and TRC1.5 pLKO.1-puro-CMV-TurboGFP™ Positive Control Transduction Particles (SHC003V and SHC003H) contain a gene encoding TurboGFP driven by the CMV (cytomegalovirus) promoter and can be useful positive controls for measuring transduction efficiency and optimizing shRNA delivery. Alternative fluorophore choices are available in the TRC1 and TRC1.5 pLKO.1-puro vector backbone. These fluorophores are also driven by the CMV promoter, and include TagCFP™ (SHC010V), TagYFP™ (SHC011V), TagRFP™ (SHC012V), and TagFP635™ (SHC013V).

Silencing of the CMV promoter may be a problem in some cell types.[8] For these cells, the Ubiquitin C promoter (UbC) can be a viable alternative.[9] Alternate promoter choices are available in the TRC1 and TRC1.5 pLKO.1-puro vector backbone. The UbC-TurboGFP (SHC014V) and UbC-TagFP635 (SHC015V) controls were generated for these types of applications. Please refer to Figure 3 for corresponding excitation and emission wavelengths.

The transduction particles containing shRNAs designed against commonly used reporter genes, TurboGFP (SHC004V and SHC004H), eGFP (SHC005V), and Luciferase (SHC007V), are useful as positive controls for knockdown and can be particularly applicable when working with stably expressing reporter cell lines. Because these shRNAs do not target any known human or mouse genes, they can also be used as non-targeting controls in many shRNA experiments.

β_2-microglobulin is a MHC Class I molecule present on most cell types.[10] It is commonly used as an endogenous control due to this universal expression. The MISSION pLKO.1-puro B2M shRNA Control Transduction Particles (SHC008V) specifically targets the human β_2-microglobulin gene and reduces expression by ~80% in A549 cells via quantitative RT-PCR analysis.

Rho GDP dissociation inhibitor (GDI) alpha (ARHGDIA) is an ubiquitously expressed protein that acts on Rho GTPases, including RhoA, Rac1, and Cdc42, by keeping these proteins in an inactive state.[11,12] Complete understanding of ARHGDIA's roles is still being elucidated but it is believed to be involved in various signal transduction pathways and cellular cytoskeleton functions. The MISSION pLKO.1-puro ARHGDIA shRNA Control Transduction Particles (SHC009V), specifically targets the human ARHGDIA gene and reduces expression by 90% or more in A549 cells, as verified by both quantitative RT-PCR and Western blot analysis using Anti-Rho-GDI, Catalog Number R3025.

The selected clones for both human positive controls were identified from the existing and available target sets for these genes because they have provided consistent knockdown, which can be useful in experimental optimization.

High titer controls SHC001H, SHC002H, SHC003H, SHC004H, and SHC016H at titers of at least 10^9 transducing units per ml (TU/ml) are designed for experiments where cell cultures require high MOI or where low volume is needed.

TRC2 Controls

The TRC2 pLKO.5-puro Empty Vector Control Transduction Particles (SHC201V) does not contain a hairpin insert and is a useful negative control that will not activate the RNA-induced silencing complex or RISC.

The TRC2 pLKO.5-puro Non-Mammalian shRNA Control Transduction Particles (SHC202V) is a negative control containing a sequence that should not target any known human or mouse gene, but will engage with RISC. This non-targeting control serves as a useful reference for interpretation of knockdown results.

The TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control Transduction Particles (SHC203V) contains a gene encoding TurboGFP driven by the CMV promoter and can be a useful positive control for measuring transduction efficiency and optimizing shRNA delivery.

Also available are the transduction particles containing shRNA to TurboGFP (SHC204V). This control is useful as a positive control for knockdown and can be particularly applicable when working with stably expressing reporter cell lines. Because this vector does not target any known human or mouse genes, it can also be used as non-targeting controls in many shRNA experiments.

Inducible Controls

Sigma offers IPTG-inducible shRNA vectors. The pLKO vector has been redesigned to contain a LacI (repressor) and a modified human U6 shRNA promoter with LacO (operator) sequences. In the absence of IPTG (isopropyl β-D-1-thiogalactoside), an analogue of lactose, LacI binds to LacO preventing expression of the shRNA. When IPTG is present, the allosteric LacI repressor changes conformation, releasing itself from lacO modified human U6 promoter, and subsequently allows expression of the shRNA.

We are proud to offer two different IPTG inducible vectors for your research. The preferred inducible vector, pLKO_IPTG_3xLacO, contains three lac operon sequences (two in the U6 promoter and one 3′ of the promoter) affording both tight regulation and great gene silencing. Whereas, the pLKO_IPTG_1xLacO vector contains a single lac operon sequence in the U6 promoter, which allows for an advantage to shRNA expression, but looser control of the promoter when not induced.

The 1X and 3X LacO Inducible Non-Target shRNA Control Transduction Particles (SHC312V and SHC332V), are negative controls containing a sequence that should not target any genes in any known species, but will engage with RISC.

The 1X and 3X LacO Inducible shRNA vectors designed against commonly used reporter genes: TurboGFP (SHC314V and SHC334V) and Luciferase (SHC317V and SHC337V) are useful as positive controls for knockdown and can be particularly applicable when working with stably expressing reporter cell lines. Because these vectors do not target any known human or mouse genes, they can also be used as non-targeting controls in many shRNA experiments.

Reagents

All controls are supplied in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin.

SHC001V, SHC002V, SHC003V, SHC004V, SHC005V, SHC007V, SHC008V, SHC009V, SHC010V, SHC011V, SHC012V, SHC013V, SHC014V, SHC015V, SHC201V, SHC202V, SHC203V, and SHC204V are provided as a 200 µL frozen stock containing at least 10^6 TU/ml.

SHC001H, SHC002H, SHC003H, SHC004H, and SHC016V are provided as a 200 µL frozen stock containing at least 10^9 TU/mL.

The MISSION Control Transduction Particles are titered via a p24 antigen ELISA assay and pg/mL of p24 are then converted to TU/ml using a conversion factor. The conversion can be viewed at: http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/mission-faqs/lentiviral-faqs.html#p24_assay.

Materials suggested but not provided

- Mammalian cells to be transduced
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- 96-well cell culture treated plates
- Puromycin dihydrochloride, cell culture tested, Catalog Number P8833
- Hexadimethrine bromide, Catalog Number H9268
- Anti-Rho-GDI, Catalog Number R3025

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 recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms for laboratory handling.[13] Follow all published RGL-2 guidelines for laboratory handling and waste decontamination. Also, use extra caution when using lentiviral transduction particles that express shRNA-targeting genes involved in cell cycle control (such as tumor suppressor genes).

Storage/Stability
All components are stable for at least 6 months after receipt when stored at –70 °C. We recommend aliquoting the material upon first thaw and avoiding repeated freeze/thaw cycles, which will severely impact titer.

Preparation Instructions
1. Prepare mammalian cell cultures so that they are growing exponentially and are no more than 70–80% confluent before transduction.
2. Prepare a stock solution of hexadimethrine bromide (Polybrene) at 2 mg/mL in water.

Procedure
The following protocol has been developed for screening in 96-well plates.

Day 1
a. Add 1.6×10^4 cells in fresh medium to the number of wells needed for each construct in a 96-well plate. Duplicate or triplicate wells for each lentiviral construct and control should be used.
b. Incubate 18–20 hours at 37 °C in a humidified incubator in an atmosphere of 5–7% CO_2.
Note: The growth rates of cells vary greatly. Adjust the number of cells plated to accommodate a confluency of 70% upon transduction. Also account for the length of time the cells will be growing before downstream analysis when determining the plating density.

Day 2
a. Remove medium from wells. To each well add 110 µL of medium and hexadimethrine bromide to a final concentration of 8 µg/mL. Gently swirl the plate to mix.
Note: Hexadimethrine bromide enhances transduction of most cell types. Some cells, like primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide to these types of cells. If working with a cell type for the first time, a hexadimethrine control only well should be used to determine cell sensitivity.
b. Add lentiviral particles at desired multiplicity of infection (MOI) to appropriate wells. Gently swirl the plate to mix. Incubate 18–20 hours at 37° C in a humidified incubator in an atmosphere of 5–7% CO_2. Cells may be incubated for as little as 4 hours before changing the medium containing lentiviral particles. Overnight incubation may be avoided when toxicity of the lentiviral particles is a concern.

Note: When transducing a lentiviral construct into a cell line for the first time, a range of volume or MOI should be tested. MOIs of 0.5, 1, 2, and 5 should be used to determine the optimal transduction efficiency and knockdown for each cell line. Transduction efficiency can be optimized using the MISSION TurboGFP Control Transduction Particles before assaying with shRNA constructs.

Multiplicity of Infection (MOI):
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:
(Total number of cells per well) × (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

Day 3
Remove the medium containing lentiviral particles from wells. Add fresh medium to a volume of 120 µL to each well.
Note: For cell types that do not strongly adhere to the plate, 100 µL of medium may be removed and replaced with 100 µL of fresh 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 titration, or Puromycin Kill Curve, in that cell line. Typically, puromycin concentrations ranging from 2–10 µg/mL are sufficient to kill most untransduced mammalian cell lines.

Incubation Time Post-Transduction

Incubation time depends on the cell line and the protein being expressed, as well as the vector construct. Non-transduced control cells under puromycin selection can be used to determine the post-transduction incubation time required to eliminate non-resistant cells for complete selection. Optimal puromycin concentration for selection should be determined by performing a titration, or Puromycin Kill Curve, in your cell line.

Puromycin Kill Curve

Prior to beginning experiments, determine the concentration of puromycin for target cells by performing a Puromycin Kill Curve.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 μL of fresh medium.
2. The next day replace medium in the wells with medium containing varying concentrations of puromycin (0, 2, 4, 6, 8, 10 μg/mL).
3. Examine viability of cells 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 medium 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.

Images

Cells that express fluorescent proteins should be imaged in a darkroom with a microscope capable of detecting fluorescence. Best images are acquired when corresponding channels are used with the microscope.

References

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7. Whither RNAi? Nature Cell Biology, **5**, 489-490 (2003).
8. Furth, P.A. et al., The variability in activity of the universally expressed human cytomegalovirus immediate early gene 1 enhancer/promoter in transgenic mice. Nucleic Acids Research, **19**, 6205-6208 (1991).
9. Schorpp, M. et al., The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice. Nucleic Acids Research, **24**, 1787-1788 (1996).
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11. Couchman, J.R. et al., RhoGDI: multiple functions in the regulation of Rho family GTPase activities. Biochem. J., **390**, 1-9 (2005).
12. Meyer, A-K. et al., Defects in cytokinesis, actin reorganization and the contractile vacuole in cells deficient in RhoGDI. EMBO, **21**, 4539-4549 (2002).
13. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (http://www4.od.nih.gov/oba)

Product Quick Reference Guide

Catalog Number Description	**Vector Backbone**	**Insert**	**Insert Sequence / Vector Description**
SHC001V **SHC001H** MISSION pLKO.1-puro Empty Vector Control	TRC1/1.5	No hairpin	No shRNA Insert
SHC201V MISSION TRC2 pLKO.5-puro Empty Vector Control	TRC2	No hairpin	No shRNA Insert
SHC002V **SHC002H** MISSION pLKO.1-puro Non-Mammalian shRNA Control	TRC1/1.5	Non human or mouse shRNA	CCGG**CAACAAGATGAAGAGCACCAA**CTC-GAG**TTGGTGCTCTTCATCTTGTTG**TTTTT
SHC202V MISSION TRC2 pLKO.5-puro Non-Mammalian shRNA Control	TRC2	Non human or mouse shRNA	CCGG**CAACAAGATGAAGAGCACCAA**CTC-GAG**TTGGTGCTCTTCATCTTGTTG**TTTTT
SHC003V **SHC003H** MISSION pLKO.1-puro-CMV-TurboGFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TurboGFP gene, under the control of the CMV promoter. TurboGFP is an improved variant of the green fluorescent protein copGFP cloned from the copepoda *Pontellina plumata*.
SHC203V MISSION TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control	TRC2	No hairpin	No shRNA insert. Contains TurboGFP gene, under the control of the CMV promoter. TurboGFP is an improved variant of the green fluorescent protein copGFP cloned from the copepoda *Pontellina plumata.*
SHC004V MISSION pLKO.1-puro TurboGFP™ shRNA Control	TRC1/1.5	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC204V MISSION TRC2 pLKO.5-puro TurboGFP™ shRNA Control	TRC2	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC005V MISSION pLKO.1-puro eGFP shRNA Control	TRC1/1.5	shRNA targeting eGFP	CCGG**TACAACAGCCACAACGTCTAT**CTC-GAG**ATAGACGTTGTGGCTGTTGTA**TTTTT
SHC007V MISSION pLKO.1-puro Luciferase shRNA Control	TRC1/1.5	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT
SHC008V MISSION pLKO.1-puro B2M shRNA Control	TRC1/1.5	shRNA targeting human β_2-microglobulin	CCGG**CAGCAGAGAATGGAAAGTCAA**CTC-GAG**TTGACTTTCCATTCTCTGCTG**TTTTT
SHC009V MISSION pLKO.1-puro ARHGDIA shRNA Control	TRC1/1.5	shRNA targeting human ARHGDIA	CCGG**CAAGATTGACAAGACTGACTA**CTC-GAG**TAGTCAGTCTTGTCAATCTTG**TTTTT
SHC010V MISSION pLKO.1-puro-CMV-TagCFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagCFP gene under the control of the CMV promoter.
SHC011V MISSION pLKO.1-puro-CMV-TagYFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagYFP gene under the control of the CMV promoter.

Product Quick Reference Guide (continued)

Catalog Number Description	Vector Backbone	Insert	Insert Sequence / Vector Description
SHC012V MISSION pLKO.1-puro-CMV-TagRFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagRFP gene under the control of the CMV promoter.
SHC013V MISSION pLKO.1-puro-CMV-TagFP635™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains FP635 gene under the control of the CMV promoter.
SHC014V MISSION pLKO.1-puro-UbC-TurboGFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TurboGFP gene under the control of the UbC promoter.
SHC015V MISSION pLKO.1-puro-UbC-TagFP635™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains FP635 gene under the control of the UbC promoter.
SHC016V **SHC016H** MISSION pLKO.1-puro Non-Target shRNA Control	TRC1/1.5	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC216V MISSION TRC2 pLKO.5-puro Non-Target shRNA Control	TRC2	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC312V MISSION 1X LacO Inducible Non-Target shRNA Control	IPTG Inducible	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC332V MISSION 3X LacO Inducible Non-Target shRNA Control	IPTG Inducible	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC314V MISSION 1X LacO Inducible TurboGFP™ shRNA Control	IPTG Inducible	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC334V MISSION 3X LacO Inducible TurboGFP™ shRNA Control	IPTG Inducible	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC317V MISSION 1X LacO Inducible Luciferase shRNA Control	IPTG Inducible	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT
SHC337V MISSION 3X LacO Inducible Luciferase shRNA Control	IPTG Inducible	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT

Control Selection Table

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 Empty Vector ControlTransduction Particles, Catalog Nos. SHC001V, SHC001H. MISSION TRC2 pLKO.5-puro Empty Vector Control Transduction Particles, Catalog No. SHC201V. MISSION pLKO.1-puro-CMV-TurboGFP™ Positive Control Transduction Particles, Catalog Nos. SHC003V, SHC003H. MISSION TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control, Catalog No. SHC203V. MISSION pLKO.1-puro-CMV-TagCFP™ Positive Control, Catalog No. SHC010V. MISSION pLKO.1-puro-CMV-TagYFP™ Positive Control, Catalog No. SHC011V. MISSION pLKO.1-puro-CMV-TagRFP™ Positive Control, Catalog No. SHC012V. MISSION pLKO.1-puro-CMV-TagFP635™ Positive Control, Catalog No. SHC013V. MISSION pLKO.1-puro-UbC-TurboGFP™ Positive Control, Catalog No. SHC014V. MISSION pLKO.1-puro-UbC-TagFP635™ Positive Control, Catalog No. SHC015V.
	These empty viral particles can serve as useful negative controls that will not activate the RNAi pathway because they do not contain an shRNA insert. It will allow for the observation of cellular effects of the transduction process. Cells transduced with the empty viral particles will provide a useful reference point for comparing specific knockdown.
Negative Control: Transduction with non-targeting shRNA	MISSION pLKO.1-puro Non-Mammalian shRNA ControlTransduction Particles, Catalog Nos. SHC002V and SHC002H. MISSION TRC2 pLKO.5-puro Non-Mammalian shRNA ControlTransduction Particles, Catalog No. SHC202V. MISSION pLKO.1-puro Non-Target shRNA Control Transduction Particles, Catalog Nos. SHC016V and SHC016H. MISSION TRC2 pLKO.5-puro Non-Target shRNA Control Transduction Particles, Catalog No. SHC216V. MISSION 1X LacO Inducible Non-Target shRNA Control Transduction Particles, Catalog No. SHC312V. MISSION 3X LacO Inducible Non-Target shRNA Control Transduction Particles, Catalog No. SHC332V.
	The Non-Target shRNA transduction particles are produced from the sequence-verified lentiviral plasmid vectors containing non-targeting shRNAs. These non-targeting shRNAs are useful negative controls that will activate RISC and the RNAi pathway, but do not target any human or mouse genes. 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 for transduction: Transduction with positive reporter viral particles	MISSION Control Transduction Particles, Catalog Nos. SHC003V, SHC003H, SHC010V, SHC011V, SHC012V, SHC013V, SHC014V, and SHC015V. MISSION TRC2-pLKO-puro CMV-TurboGFP, Catalog No. SHC203V.
	These are useful positive controls for measuring transduction efficiency and optimizing shRNA delivery.
Positive Controls for knockdown: Transduction with shRNA targeting report gene	MISSION TurboGFP shRNA Control Transduction Particles, Catalog Nos. SHC004V, SHC004H. MISSION TRC2-pLKO-puro TurboGFP shRNA Control Transduction Particles, Catalog No. SHC204V. MISSION 1X LacO Inducible TurboGFP™ shRNA Control Transduction Particles, Catalog No. SHC314V. MISSION 3X LacO Inducible TurboGFP™ shRNA Control Transduction Particles, Catalog No. SHC334V.
	The TurboGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–puro vector containing shRNA that targets TurboGFP (Catalog No. 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 HEK293T 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.
	MISSION eGFP shRNA Control Transduction Particles, Catalog Number SHC005V
	The eGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–puro vector containing shRNA that targets eGFP (Catalog No. SHC005), and can be used as a positive control to quickly visualize knockdown. These eGFP (GenBank Accession No. pEGFP U55761) shRNA transduction particles are also useful as a negative non-target control because the shRNA does not target any human or mouse genes.

Control Selection Table (continued)

Recommended Control	Objective
Positive Controls for knockdown: Transduction with shRNA targeting report gene (continued)	MISSION Luciferase shRNA Control Transduction Particles, Catalog No. SHC007V. MISSION 1X LacO Inducible Luciferase shRNA Control Transduction Particles, Catalog No. SHC317V. MISSION 3X LacO Inducible Luciferase shRNA Control Transduction Particles, Catalog No. SHC337V.
	The MISSION Luciferase shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1-puro vector containing an shRNA insert that targets luciferase (Catalog Number SHC007) from North American Firefly, *Photinus pyralis* (GenBank Accession No. M15077). These transduction particles can be used as a positive control to quickly confirm knockdown. Because the shRNA targets firefly luciferase, and it does not target any human or mouse genes, it can also be used as a negative non-target control in shRNA experiments.
Positive Controls for knockdown: Transduction with shRNA targeting gene	MISSION shRNA Human Positive Control Vector #1 Transduction Particles, Catalog No. SHC008V.
	The β_2-microglobulin shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–Puro vector containing shRNA that targets human β_2-microglobulin (Catalog No. SHC008). This control will provide clear and measurable knockdown of the human target, typically 80–90% in A549 cells, a human epithelial lung carcinoma cell line.
	MISSION shRNA Human Positive Control Vector #2 Transduction Particles, Cat. No. SHC009V.
	The ARHGDIA shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–Puro vector containing shRNA that targets human Rho GDP Dissociation Inhibitor alpha (Catalog No. SHC009). This control will provide clear and measurable knockdown of the human target, typically 80–90% in A549 cells, a human epithelial lung carcinoma cell line.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solutions
Low level 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.
	MOI is too low.	Transduce at a higher MOI.
	Cells were harvested and assayed too soon after transduction.	The shRNA must be permitted to accumulate in cells. Harvest 48–72 hours after transduction.
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.
Cytotoxic effects observed after transduction.	Hexadimethrine bromide was used during transduction.	Be sure that cells are not sensitive to hexadimethrine bromide. Omit the hexadimethrine bromide during the transduction.
No fluorescent protein detected	Cells need more time to express the fluorescent protein	Protein expression times are cell line dependent; continue viewing fluorescence daily with media changes as needed. Approximately 6 days may be needed to view protein expression.
	Cells need to be imaged in a darkroom	Cells that express fluorescent proteins should be imaged in a darkroom with a microscope capable of detecting fluorescence. Best images are acquired when corresponding channels are used with the microscope.

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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

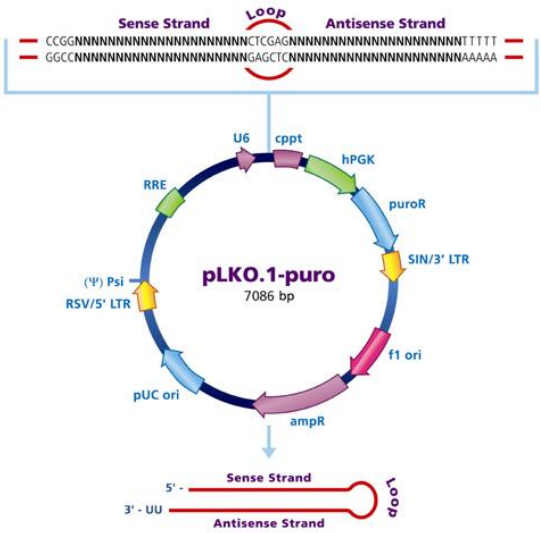


Figure 2. TRC2 Lentiviral Plasmid Vector TRC2-pLKO-puro Features

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

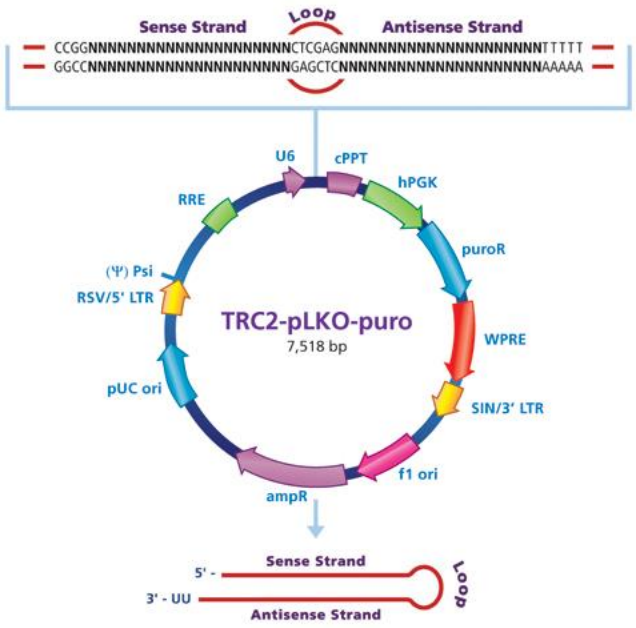


Figure 3.
Inducible shRNA Vectors

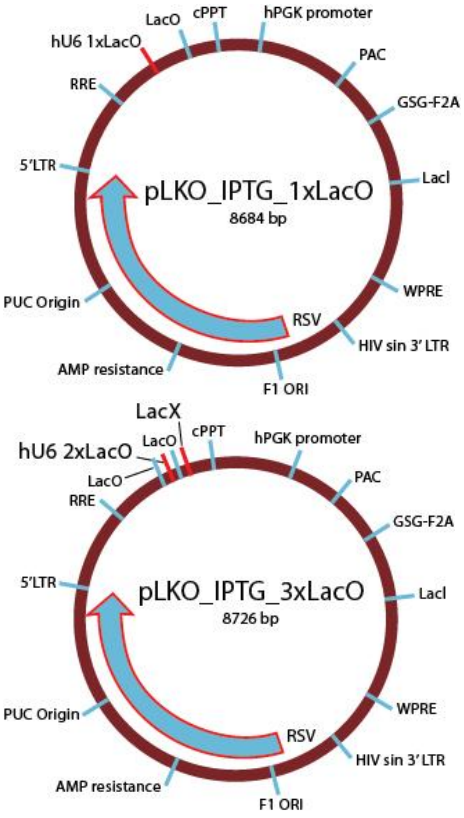
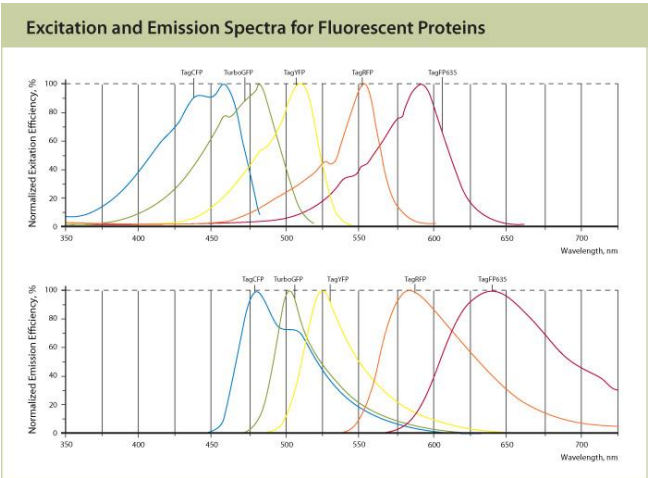


Figure 4.
Excitation and Emission Wavelengths for Fluorescent Proteins

Catalog Number	Fluorophore	Excitation (nm)	Emission (nm)
SHC003/SHC014	TurboGFP	482	502
SHC010	TagCFP	458	480
SHC011	TagYFP	508	524
SHC012	TagRFP	555	584
SHC013/SHC015	TagFP635	588	635



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