

## Product Information

### MISSION® shRNA Control Vectors

Catalog Numbers **SHC001, SHC002, SHC003, SHC004, SHC005, SHC007, SHC008, SHC009, SHC010, SHC011, SHC012, SHC013, SHC014, and SHC015**

Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Small interfering RNAs (siRNAs) generated from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) 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 delivered by 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.

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, found in MISSION Lentiviral Packaging Mix, Catalog Number SHP001.<sup>1,2</sup> 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<sup>3</sup>, overcoming low transfection and integration difficulties when using these cell types.

When conducting experiments using MISSION shRNA clones, 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 Control Vectors are lentiviral-based vectors that are useful as both positive and negative controls in experiments using the MISSION shRNA library. The DNA format controls may be used in direct transfection of target cells or they may also be used to create replication-incompetent viral particles.

Figure 1 depicts the base vector for all TRC1 and TRC1.5 clones. The pLKO.1-puro empty vector, Catalog Number SHC001, does not contain a hairpin insert, and is a useful negative control that will not activate the RNA-induced silencing complex, or RISC. The pLKO.1-puro Non-Target shRNA Control Vector, Catalog Number SHC002, is a negative control containing a sequence that should not target any known human or mouse gene, but will engage with RISC. This is a useful reference for interpretation of knockdown.

The MISSION pLKO.1-puro CMV-TurboGFP™ Control Vector, Catalog Number SHC003, contains a gene encoding TurboGFP driven by the CMV promoter, and can be a useful positive control for measuring transfection efficiency and optimizing shRNA delivery. Alternative fluorophore choices are available, also driven by the CMV promoter. These include TagCFP™ (SHC010), TagYFP™ (SHC011), TagRFP™ (SHC012) and TagFP635™ (SHC013).

Silencing of the CMV promoter may be a problem in some cell types. For these cells, the ubiquitin promoter (UbC) can be a viable alternative. The pLKO.1-puro UbC-TurboGFP (SHC014) and pLKO.1-puro UbC-TagFP635 (SHC015) controls were generated for these types of applications. Please refer to Figure 2 for corresponding excitation and emission wavelengths.

The shRNA vectors designed against commonly used reporter genes: TurboGFP (SHC004), eGFP (SHC005), and Luciferase (SHC007), 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.

$\beta_2$ -microglobulin is a MHC Class I molecule present on most cell types.<sup>5</sup> It is commonly used as an endogenous control due to this universal expression. The MISSION shRNA Human Positive Control Vector #1 Purified DNA, Catalog Number SHC008, specifically targets the human  $\beta_2$ -microglobulin gene and reduces expression by approximately 80% in A549 cells via quantitative RT-PCR analysis.

Rho GDP dissociation inhibitor (GDI) alpha (ARHGDI) is an ubiquitously expressed protein that acts on Rho GTPases, including RhoA, Rac1, and Cdc42, by keeping these proteins in an inactive state.<sup>6,7</sup> Complete understanding of ARHGDI's roles is still being elucidated but it is believed to be involved in various signal transduction pathways and cellular cytoskeletal functions. The MISSION shRNA Human Positive Control Vector #2 Purified DNA, Catalog Number SHC009, specifically targets the human ARHGDI gene and reduces expression by 90% or more in A549 cells, 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.

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.<sup>4</sup> 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.

### Components/Reagents

Each MISSION Control Vector is provided as 10  $\mu$ g purified plasmid DNA at a concentration of ~500 ng/ $\mu$ L in 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA.

### 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

These products are guaranteed to be stable for at least one year after receipt when stored at  $-20$  °C.

### Materials suggested but not provided

- Mammalian cells to be transfected or transduced
- ESCORT™ II Transfection Reagent, Catalog Number L6037
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- Puromycin dihydrochloride, cell culture tested, Catalog Number P8833
- MISSION Lentiviral Packaging Mix, Catalog Number SHP001
- Anti-Rho-GDI, Catalog Number R3025

### Procedures

#### Transfection

Transfection reagents that exhibit high performance in delivery of plasmid DNA are recommended. Sigma offers ESCORT™ II Transfection Reagent, Catalog Number L6037.

Seed cells and transfect according to the transfection reagent manufacturer's instructions. Cells should be healthy, free of contamination, proliferating well, and plated at an appropriate density.

#### Incubation Time Post-Transfection

Incubation time depends on the cell line and the protein being expressed, as well as the vector construct. Untransfected control cells under puromycin selection can be used to determine the post-transfection 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.

#### Lentiviral Production

Controls may be co-transfected in packaging cells (HEK293T) with MISSION Lentiviral Packaging Mix (Catalog Number SHP001) to produce self-inactivating replication incompetent viral particles. Seed cells and co-transfect according to the MISSION Lentiviral Packaging Mix Technical Bulletin.

### Puromycin Kill Curve

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

1. Plate  $1.6 \times 10^4$  cells into wells of a 96-well plate with 120  $\mu$ L of fresh medium.
2. The next day replace the medium in the wells with medium containing varying concentrations of puromycin (0, 2, 4, 6, 8, 10  $\mu$ g/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.

### **Images**

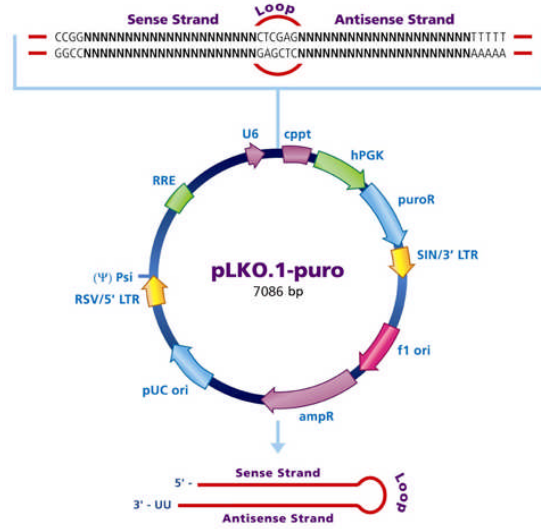
Cells that express fluorescent proteins should be imaged in a darkroom with a microscope capable of detecting fluorescence. Best images are acquired when corresponding channels are used with the microscope.

### **References**

1. Zufferey, R., et al., Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* **15**, 871-85 (1997).
2. Zufferey, R., et al., Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J Virol.*, **72**, 9873-80 (1998).
3. Stewart, S.A., et al., Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
4. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).
5. Schardijn, Gus H.C., and L.W. Stadius Van Eps,  $\beta_2$ -microglobulin: Its significance in the evaluation of renal function. *Kidney International*, Vol. 32, pp. 635-641 (1987).
6. Couchman, J.R., et al., RhoGDI: multiple functions in the regulation of Rho family GTPase activities. *Biochem J.*, **390**, 1-9 (2005).
7. Meyer, A-K, et al., Defects in cytokinesis, actin reorganization and the contractile vacuole in cells deficient in RhoGDI. *EMBO* **21(17)**, 4539-4549 (2002).

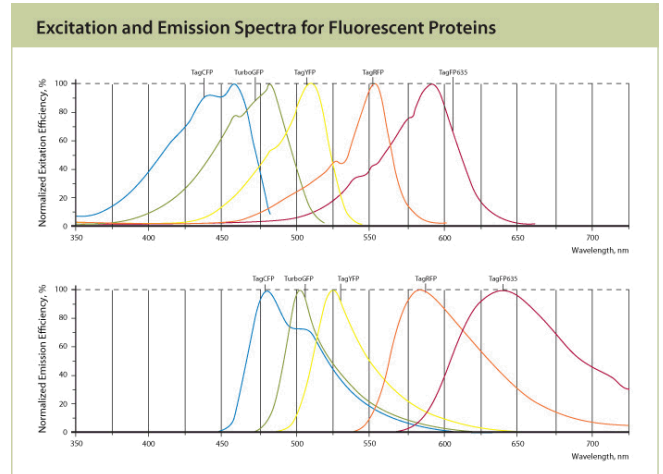
**Figure 1. TRC1 and TRC1.5 Lentiviral Plasmid Vector pLKO.1-puro Features**

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. 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



## Product Quick Reference Guide

Catalog Number	Insert	Insert Sequence
Description		
<b>SHC001</b>		
MISSION pLKO.1-puro Control Vector	No hairpin	No Insert
<b>SHC002</b>		
MISSION Non-Target shRNA Control Vector	Non human or mouse shRNA	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTTTCATCTTGTTGTTTT
<b>SHC003</b>		
MISSION pLKO.1-puro CMV-TurboGFP	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 <i>Pontellina plumata</i> .
<b>SHC004</b>		
MISSION TurboGFP shRNA Control Vector	shRNA targeting TurboGFP	CCGGCGTGATCTTCACCGACAAGATCTCGAGATCTTGTGGTGAAGATCACGTTTTT
<b>SHC005</b>		
MISSION eGFP shRNA Control Vector	shRNA targeting eGFP	CCGGTACAACAGCCACAACGTCTATCTCGAGATAGACGTTGTGGCTGTTGATTTTT
<b>SHC007</b>		
MISSION Luciferase shRNA Control Vector	shRNA targeting Luciferase	CCGGCGCTGAGTACTTCGAAATGTCCTCGAGGACATTTGAAGTACTCAGCGTTTTT
<b>SHC008</b>		
MISSION shRNA Human Positive Control Vector #1 Purified DNA	shRNA targeting human $\beta_2$ -microglobulin	CCGGCAGCAGAGAATGGAAAGTCAACTCGAGTTGACTTTCATTCTCTGCTGTTTTT
<b>SHC009</b>		
MISSION shRNA Human Positive Control Vector #2 Purified DNA	shRNA targeting human ARHGDI	CCGGCAAGATTGACAAGACTGACTACTCGAGTAGTCAGTCTTGTCAATCTTGTTTTT
<b>SHC010</b>		
MISSION Control Vector pLKO.1-puro CMV-TagCFP	No hairpin	No shRNA insert. Contains TagCFP gene under the control of the CMV promoter.
<b>SHC011</b>		
MISSION Control Vector pLKO.1-puro CMV-TagYFP	No hairpin	No shRNA insert. Contains TagYFP gene under the control of the CMV promoter.
<b>SHC012</b>		
MISSION Control Vector pLKO.1-puro CMV-TagRFP	No hairpin	No shRNA insert. Contains TagRFP gene under the control of the CMV promoter.
<b>SHC013</b>		
MISSION Control Vector pLKO.1-puro CMV-TagFP635	No hairpin	No shRNA insert. Contains FP635 gene under the control of the CMV promoter.
<b>SHC014</b>		
MISSION Control Vector pLKO.1-puro UbC-TurboGFP	No hairpin	No shRNA insert. Contains TurboGFP gene under the control of the UbC promoter.
<b>SHC015</b>		
MISSION Control Vector pLKO.1-puro UbC-TagFP635	No hairpin	No shRNA insert. Contains FP635 gene under the control of the UbC promoter.

## Control Selection Table

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	<p>MISSION Control Vector pLKO.1-puro, Catalog No. SHC001.  MISSION Control Vector pLKO.1-puro CMV-TurboGFP, Catalog No. SHC003.  MISSION Control Vector pLKO.1-puro CMV-TagCFP, Catalog No. SHC010.  MISSION Control Vector pLKO.1-puro CMV-TagYFP, Catalog No. SHC011.  MISSION Control Vector pLKO.1-puro CMV-TagRFP, Catalog No. SHC012.  MISSION Control Vector pLKO.1-puro CMV-TagFP635, Catalog No. SHC013.  MISSION Control Vector pLKO.1-puro UbC-TurboGFP, Catalog No. SHC014.  MISSION Control Vector pLKO.1-puro UbC-TurboFP635, Catalog No. SHC015.</p> <p>These vectors can serve as useful negative controls that will not activate the RNAi pathway because they do not contain an shRNA insert. They will allow for observation of cellular effects of the transfection process. Cells transfected with these vectors provide a useful reference point for comparing specific knockdown.</p>
Negative Control: Transfection with non-targeting shRNA	<p>MISSION Non-Target shRNA Control Vector, Catalog No. SHC002.</p> <p>The Non-Target shRNA vector is produced from the sequence-verified lentiviral plasmid, pLKO.1-puro vector containing a non-targeting shRNA (Catalog Number SHC002). This non-targeting shRNA is a useful negative control that should activate RISC and the RNAi pathway, but should not target any known 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.</p>
Positive Control for transfection: Transfection with positive reporter viral particles	<p>MISSION Control Vectors, Catalog Nos. SHC003, SHC010, SHC011, SHC012, SHC013, SHC014, and SHC015.</p> <p>These are useful positive controls for measuring transfection efficiency and optimizing shRNA delivery.</p>
Positive Controls for knockdown: Transfection with shRNA targeting reporter gene	<p>MISSION TurboGFP shRNA Control Vector, Catalog No. SHC004.</p> <p>The TurboGFP shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets TurboGFP (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 known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.</p> <p>MISSION eGFP shRNA Control Vector, Catalog No. SHC005.</p> <p>The eGFP shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets eGFP. Because this shRNA targets eGFP (GenBank Accession No. pEGFP U55761), and it does not target any known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.</p> <p>MISSION Luciferase shRNA Control Vector, Catalog No. SHC007.</p> <p>The MISSION Luciferase shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets the luciferase from North American Firefly, <i>Photinus pyralis</i> (GenBank Accession No. M15077). Because the shRNA targets firefly luciferase, and it does not target any known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.</p>
Positive Controls for knockdown: Transfection with shRNA targeting gene	<p>MISSION shRNA Human Positive Control Vector #1, Catalog No. SHC008.</p> <p>The <math>\beta_2</math>-microglobulin shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1-puro vector containing shRNA that targets human <math>\beta_2</math>-microglobulin (Catalog Number 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.</p> <p>MISSION shRNA Human Positive Control Vector #2, Catalog No. SHC009.</p> <p>The ARHGDI<math>\alpha</math> shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1-puro vector containing shRNA that targets human Rho GDP Dissociation Inhibitor <math>\alpha</math> (Catalog Number 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.</p>

## Troubleshooting Guide

Problem	Possible Cause	Suggested Solutions
Low transfection efficiency	Volume of transfection cocktail	For optimization, compare transfection performance when different volumes of transfection cocktail are added to the wells (e.g., 75, 100, 120, and 150 $\mu\text{L}/\text{well}$ ).
	Contaminated DNA	Use a high-quality plasmid preparation method yielding an $\text{OD}_{260/280} = 1.8\text{--}1.85$ .
		Use endotoxin free DNA. For endotoxin removal, use Endotoxin Removal Solution, Catalog Number E4274.
	Sub-optimal DNA/Transfection Reagent ratio	Transfection efficiency may be increased by changing the ratio of $\mu\text{g DNA}/\mu\text{L}$ transfection reagent.
	Vector used	In order to achieve an optimal expression rate of the transfected gene, the promoter should be compatible with the cell type.
		Low transfection efficiency results in low expression rates. On the other hand, very high exogenous protein expression levels may be cytotoxic.
		Perform a control transfection.
Cell growth conditions	If cells have a high passage number, start a new culture from stocks of a lower passage number	
	See that cells were not dramatically stressed during plating procedure or while incubated. See that the medium and serum used are optimal for cell growth.	
	Check for the presence of mycoplasma in the cells.	
Assay	Ensure that the cells are plated at the optimal density.	
	Use a positive control to ensure that the assay works properly.	
Signs of cell cytotoxicity	Expressed protein is toxic to the cells at the current expression level.	If the particular cell type is obligatory, try to express the gene under a different promoter
	Volume of transfection cocktail	For optimization, compare transfection performance when different volumes of transfection cocktail are added to the wells (e.g., 75, 100, 120, and 150 $\mu\text{L}/\text{well}$ ). Substitute the medium containing the transfection cocktail with fresh medium 6–24 hours post transfection
	Contaminated DNA	Use a high-quality plasmid vector.
		For endotoxin free DNA, use Endotoxin Removal Solution, Catalog No. E4274.
	Cells are stressed	Ensure that cells are not dramatically stressed during plating procedure or while incubated
Mycoplasma contamination	Check for the presence of mycoplasma in the cells.	
Transfection efficiency varies between repeats within the same experiment	Cell density and incubation conditions	The density of the cells in the different wells could vary due to clump formation or seeding cells without mixing. Avoid clump formation following trypsinization by repeatedly pipetting the cells. Verify that the plate placed in the incubator is perfectly horizontal and not adjacent to the incubator wall.
	Mycoplasma contamination	Prepare new cells.
	Cell passage number too high	Prepare new cells.
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.
	Transfection of DNA	Sigma recommends producing virus from the DNA fluorescent protein control vectors in order to view fluorescence post-transduction.

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