shRNA Technologies

The industry’s broadest, most technologically advanced portfolio of shRNA reagents for transient, long-term, inducible and in vivo RNA interference.

Which shRNA reagent is best for you?

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Formats

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- Learn more on page 35
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- Learn more on page 37

Follow the icons to your product solution

- Bacterial glycerol stock
  - Bacterial culture transformed with a plasmid vector, grown in the presence of glycerol for freezer storage and supplied in single tubes. Available as individual clones, target gene sets (at least three constructs per target gene) and pre-defined or custom libraries.
- Arrayed library format
  - Genome-scale, pre-defined or custom libraries of 96-well microtiter plates.
- Gene families and pathways
  - Pre-defined libraries arranged by gene family and/or function and available in 96-well microtiter plates.
- High-titer viral particles
  - Transduction-ready concentrated lentiviral particles supplied in tubes.
- shRNA Starter Kit
  - All reagents and protocols necessary for starting a gene silencing experiment are provided in a single, convenient package (see p. 36 for more details).
- Fluorescent markers
  - Visually track expressed shRNA with TurboGFP® (green fluorescent protein) or TurboRFP® (red fluorescent protein).
- Species
  - Human
  - Mouse
  - Rat

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Viral particles are shipped on dry ice

Perform transient or long-term gene silencing, even in difficult-to-transfect cell types

Achieve specific knockdown with minimal toxicity

High-titer purified lentiviral particles for efficient transduction

Study essential gene function with inducible shRNA

Find out more on pages 32-34

Find out more on pages 30-31

Find out more on page 39

Find out more on pages 34 and 36

* For GIPZ and TRIPZ shRNA, at least one out of three shRNA constructs is guaranteed to reduce target mRNA levels by 70% or more when used in combination with the non-targeting control and the GAPDH positive control.

Find out more on page 32-34

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Less toxicity, more specificity with microRNA-adapted shRNA

microRNA-adapted shRNAs offer a clear advantage over simple hairpin shRNAs. All shRNA designs embed the gene-specific silencing sequence in a primary microRNA transcript, thus creating a RNAi trigger that silences genes with increased specificity.

microRNA-based shRNAs exhibit lower cellular toxicity and off-target effects

- Simple stem-loop designed hairpins driven by strong promoters may cause cellular toxicity. Reducing the multiplicity of infection (MOI) can be insufficient to decrease the toxic effects potentially caused by the accumulation of transcripts. Published findings show a significant reduction in neurotoxicity could be attributed to moving the silencing sequence from a simple hairpin to a microRNA scaffold.  

- Simple stem-loop hairpins may disrupt normal microRNA processing and function, causing cell death and off-target effects. Published findings suggest that the U6 promoter may interact with certain cancer-causing genes to accelerate tumorigenesis and cause toxicity.  

- Off-target silencing resulting in measurable phenotypic effects were observed in vitro following transduction of simple stem-loop hairpin shRNA for several genes tested. In addition, persistent disruption of microRNA biogenesis was observed for six critical cellular microRNAs, causing 60-80% suppression of native microRNAs and up to three-fold up-regulation of putative target genes.  

Borrowing the endogenous microRNA pathway to induce RNAi

dRNA approaches include the introduction of genetically engineered viral vectors or plasmid-based vectors expressing silencing sequences embedded in an endogenous microRNA scaffold (1) or simple stem-loop shRNA (2). Expressed sequences (1 and 2, shown in blue) enter the endogenous pathway at an early stage and are efficiently processed into potent silencing molecules using the endogenous microRNA mechanism (see p. 9). All of these approaches lead to target mRNA cleavage (shown in purple) and gene silencing.

References
SMARTchoice™ Lentiviral shRNA

### Availability
- **SMARTchoice shRNA Promoter Selection Plate**
- Custom manufactured high-titer lentiviral particles

**Catalog No.**
- SMARTchoice Promoter Selection Plate: SP-001000-01

**SMARTchoice Lentiviral shRNA**
- Individual Lentiviral Particles: 100 or 200 µL
- Set of 5 Lentiviral Particles: 100 or 200 µL

- Estimated manufacturing time is four to six weeks

**Related products**
- SMARTchoice shRNA Controls
  - See p. 57

### Description
To fully harness the utility of lentiviral vector approaches in shRNA-mediated gene silencing, careful attention must be paid to the choice of promoter controlling its expression. Inefficient promoter activity due to varying cellular and biological contexts in sub-optimal knockdown and can be misinterpreted as poor shRNA functionality. The SMARTchoice shRNA platform permits the researcher to first observe activity of multiple promoters in the target cells of interest and then order SMARTvector gene-specific lentiviral shRNA and controls with optimal promoter choices, saving time and money by making informed decisions.

### Benefits
- **SMARTchoice shRNA Promoter Selection Plate** allows straightforward identification of optimal promoters in cells of interest.
- Custom SMARTchoice vectors based on advanced microRNA-adapted shRNA design for specific silencing and minimal off-target effects.
- Flexibility to order gene-specific shRNA vectors with choice of seven different promoters and two fluorescent reporters to maximize success.

#### Functional data

**Serial Dilutions**

- **A549**
- **HEK293T**
- **Jurkat**

**Make informed decisions in the design of gene silencing experiments using the SMARTchoice shRNA Promoter Selection Plate.** Human A549, HEK293T and Jurkat cells were transduced with lentiviral particles arrayed in the SMARTchoice shRNA Promoter Selection Plate. TurboGFP expression was assessed by fluorescence microscopy 72 hours post-transduction. Images clearly demonstrate that the most functional promoter in the A549 cells is mEF1α, whereas the hCMV promoter is most active in HEK293T, and the mEF1α promoter is most active in Jurkat cells.

**Dharmacon Process**

1. **SMARTchoice Promoter Selection Plate**
   - Argoed viral particles
   - Normalized titers

2. Transduce cells of interest
   - Cells specific to your application

3. Select optimal promoter
   - Based on fluorescence intensity

4. **shRNA vector construction**
   - SMARTchoice promoter and fluorescent reporter of choice

5. Lentiviral particle production
   - High-titer particles
   - Transduction-ready

6. Perform gene silencing experiments
   - Utilizing matched controls

**SMARTchoice experimental workflow:** Identify the ideal promoter for your cells of interest with the transduction-ready SMARTchoice shRNA Promoter Selection Plate. Next, place an online order for SMARTchoice Lentiviral shRNA particulars with your choice of promoter and reporter and save the extensive time, labor and money required for production of high-titer lentiviral particles in your lab. Benefits from our internal quality-controlled process of cloning and packaging your customized vector into lentiviral particles using packaging technology for enhanced biosafety.
SMARTchoice™ Inducible Lentiviral shRNA

Description
Combining all of the advantages of the SMARTvector shRNA design innovations with the recently developed Tet-On 3G tet-off inducible expression system and the flexibility of SMARTchoice promoter and reporter options, this novel single-vector regulatable RNAi system permits the rapid development of stable cells with tightly controlled shRNA expression.

Benefits
- Advanced universal scaffold and proprietary shRNA design algorithm for potent silencing at single-copy integration and minimal off-target effects
- Tight control of shRNA and reporter gene expression utilizing the latest generation Tet-inducible expression technology, the Tet-On® 3G Inducible System
- Unmatched flexibility with your choice of two reporters and four promoters in an inducible system

Elements of the SMARTchoice Inducible shRNA lentiviral backbone.
The SMARTchoice inducible vector customization options include four constitutive promoter and two reporter options. While the inducible control of the SMARTvector shRNA and the reporter gene are conferred by the Tet 3G promoter (Ptet3G), the expression of the Tet-On® 3G transactivator protein is under the control of a constitutive RNA pol II promoter. The SMARTchoice Inducible shRNA constitutive RNA pol II promoter options include: mCMV (mouse cytomegalovirus promoter); intermediate early promoter; PGK (mouse phosphoglycerate kinase promoter); mEF1α (mouse elongation factor 1 alpha promoter); and hEF1α (human elongation factor 1 alpha promoter). Fluorescent reporter options are TurboGFP® and puromycin.

SMARTchoice Inducible shRNAs enable tightly controlled knockdown of essential genes in a time- and dose-dependent manner.
(A) U2OS cells were transduced at MOI = 0.1 with SMARTchoice Inducible mCMV vectors carrying either a non-targeting control shRNA (NTC) or on shRNA directed against the ubiquitin B (UBB) gene. Cells were selected for 3 days with 1.5 μg/mL puromycin. After selection, cells were seeded on 2000 cells per well in 96-well plates. 24 hours later (Day 1), shRNA expression was induced with the indicated dose of doxycycline, and cell number was then measured each day with the Cell Titer-Glo® assay (Promega). Each data point represents the mean ± SD of triplicate wells. (B) SMARTchoice Inducible lentiviral shRNA with mCMV cell proliferation assay. (C) SMARTchoice Inducible lentiviral shRNA with mCMV cell proliferation assay.

Availability
- Individual shRNAs and sets of three
- Controls: non-targeting and positive (PuR.R and GAPDH)
- Use the SMARTchoice inducible non-targeting A-4 pack to determine the optimal promoter for your experiment

Catalog No.*
- SMARTchoice Inducible Lentiviral shRNA Particles
  - SMARTchoice Inducible Lentiviral shRNA: 100 or 200 μL, 10^7 TU/mL
  - Set of 3 SMARTchoice Inducible Lentiviral shRNA: 100 or 200 μL, 10^7 TU/mL

*These are generic product identifiers. Actual catalog numbers will be gene specific, reporter and promoter specific, e.g., SMARTchoice Inducible Lentiviral shRNA (GAPDH-1,2,3,4). Estimated manufacturing time is four to six weeks.

GIPZ™ Lentiviral shRNA

Description
Perform specific and efficient, transient or long-term RNAi with GIPZ Lentiviral shRNA. Human and mouse genes are each targeted with multiple shRNA constructs that have been cloned into the pGIPZ lentiviral vector.

Benefits
- Efficient and guaranteed* knockdown
- TurboGFP® tracks shRNA expression
- Lentiviral delivery extends RNAi to primary and non-dividing cells
- Bacterial glycerol stocks or purified, high-titer lentiviral particle format**

Functional data

Vector Element Utility
TurboGFP® Human cytoskeletal protein promoter drives strong transgene expression
Psi packaging sequence allows viral genome packaging using lentiviral packaging systems
IRES Internal ribosomal entry site allows expression of TurboGFP and puromycin resistance genes in a single transcript

*Estimated manufacturing time is four to six weeks.
**At least one out of three constructs is guaranteed to reduce target shRNA levels by 70% or more when used in combination with the non-targeting control and the GAPDH positive control.

Related products
Turbofect Transfection Reagent
- See p. 62

GIPZ and TRIPZ shRNA Starter Kits
- See p. 38

Trans-Lentiviral shRNA Packaging Kit
- See p. 63

GIPZ shRNA Controls
- See p. 57

Pre-defined and Custom shRNA Libraries
- See p. 68

SMARTchoice shRNA; 100 or promoter-specific. (SMARTchoice Inducible shRNA experiment

See p. 57

Cell density, defined as number of nuclei per field was computed for 18 fields per condition.

Relationship between knockdown and reporter expression

Effective knockdown using GIPZ lentiviral shRNA. OSCAR-B cells were transduced with GIPZ lentiviral shRNA constructs at MOI = 0.4-2 in 24-48 hour biologic replicates. Cells were puromycin-selected (50 μg/mL) starting 48 hours post-transduction. RNA was isolated 48 hours post-transduction. qPCR was performed in triplicate via TaqMan® Gene Expression Assays using 18S RNA as an internal reference. On average two out of these shRNA produced greater than 70% knockdown compared to the GIPZ Non-targeting Control shRNA.
**TRIPZ™ Inducible Lentiviral shRNA**

**Description**
Study the function of essential genes, validate cellular phenotypes and generate regulated knockdown cell lines with TRIPZ Inducible Lentiviral shRNA.

**Benefits**
- Regulatable and reversible gene silencing
- Guarantee knockdown
- Tet-On or Tet-Off configurations
- TurboGFP tracks induced shRNA expression

**Functional data**
- pTRIPZ
- WPRE
- RRE
- Ψ
- IRES
- 3' SIN LT R
- Pur oR
- WPRE
- pU Co ri
- SV 40 or i
- BlastR
- SV 40 or i
- tGFPnu c
- WPRE
- Pu roR
- SV 40 or i
- BlastR
- WPRE
- *At least one out of three constructs is guaranteed to reduce target shRNA levels by ≥70% or more when used in combination with the non-targeting control and the dox-induced positive control.

**Vector Element | Utility**
- TRE | Tetracycline-inducible promoter
- tRFP | TurboGFP reporter for visual tracking of transduction and shRNA expression
- shRNA | microRNA-adopted shRNA
- hCMV | Human cytomegalovirus promoter
- UBC | Human ubiquitin C promoter
- rTAS | Reverse tetracycline-transactivator 3 for tetracycline-dependent induction of the TRE promoter
- PurO | Purmycin resistance permits antibiotic-selective pressure and propagation of stable integrants
- IRES | Internal ribosomal entry site allows expression of rTAS and purmycin resistance genes in a single transcript
- 5' LTR | 5'-long terminal repeat
- 3' SIN LTR | 3'-self-inactivating long terminal repeat; for increased lentivirus safety
- U6 | Pol packaging sequence allows viral genome packaging using lentiviral packaging systems
- WPRE | Enhances packaging efficiency of full-length viral genomes
- RE | Reverse response element enhances titre by increasing packaging efficiency of full-length viral genomes
- pTRIPZ

**Availability**
- Individual clone and target gene set
- shRNA Starter kit
- Libraries: pre-defined and custom
- Controls: empty vector, non-targeting and positive

**Related products**
- TurboFlex Transfection Reagent
- PP2 and TRIP shRNA
- Starter Kit
- Trans-Lentiviral shRNA Packaging Kit
- TRIP shRNA Controls
- Pre-defined and Custom shRNA Libraries

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**TRC Lentiviral shRNA**

**Description**
The TRC Lentiviral shRNA Library was developed by the Broad Institute of MIT and Harvard University. This collection targets both human and mouse genes with broad coverage per transcript.

**Benefits**
- Simple stem-loop shRNA design
- Amenable to in vitro and in vivo applications
- Lentiviral vector enables transduction of primary and non-dividing cell lines

**Functional data**
- pTRIPZ
- WPRE
- RRE
- Ψ
- IRES
- 3' SIN LT R
- Pur oR
- WPRE
- pU Co ri
- SV 40 or i
- BlastR
- SV 40 or i
- tGFPnu c
- WPRE
- Pu roR
- SV 40 or i
- BlastR
- WPRE

**Vector Element | Utility**
- USE | Human U6 RNA polymerase III promoter provides high level of expression in the target cells
- shRNA | Simple stem-loop shRNA for gene knockdown
- hCMV | Human cytomegalovirus promoter drives expression of the purmycin resistance gene
- PurO | Purmycin resistance permits antibiotic-selective pressure and propagation of stable integrants
- RRE/LTR | RRE promoter/T long terminal repeat promotes strong lentiviral transcription in the packaging cells in the absence of Tat
- SIN LTR | Self-inactivating long terminal repeat for increased lentivirus safety
- WPRE | Enhances packaging efficiency of full-length viral genomes

**Availability**
- Individual shRNA clones and target gene sets
- Libraries: pre-defined and custom
- Controls: empty vector and positive

**Related products**
- TurboFlex Transfection Reagent
- Trans-Lentiviral shRNA Packaging Kit
- TRC shRNA Controls
- Pre-defined and Custom shRNA Libraries

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**Induced knockdown at low MOI**

HEK293T cells were transduced with various TRIPZ lentiviral shRNA at a MOI of 0.3, purmycin-selected (15 μg/ml) at 48 hours and induced with doxycycline (1 μg/ml) starting 5 days post-transduction. RNA was isolated 2 weeks post-transduction and knockdown of indicated target genes was compared to the Non-targeting TRIPZ Lentiviral shRNA-Control (NS1) as determined by TaqMan® Gene Expression Assays. Each bar represents an experimental duplicate while qPCR was performed in triplicate using U6 RNA as an internal reference.

**References**
GIPZ™ and TRIPZ™ shRNA Starter Kits

Availability

<table>
<thead>
<tr>
<th>Description</th>
<th>Catalog No.</th>
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<tr>
<td>GIPZ shRNA Lentiviral shRNA</td>
<td>RHS4287</td>
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<tr>
<td>GIPZ shRNA transfection Starter Kit</td>
<td>RHS5086</td>
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<tr>
<td>GIPZ shRNA transduction Starter Kit</td>
<td>RHS4741</td>
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<tr>
<td>TRIPZ shRNA Lentiviral shRNA</td>
<td>RHS5087</td>
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<tr>
<td>TRIPZ shRNA transfection Starter Kit</td>
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<tr>
<td>TRIPZ shRNA transduction Starter Kit</td>
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**Description**

The shRNA Starter Kits combine the versatility and specificity of lentiviral shRNA, with the simplicity of validated controls and optimized delivery reagents to provide a complete set of reagents for your RNAi experiment with guaranteed silencing.

Each kit contains:

- Multiple human or mouse, GIPZ or TRIPZ shRNAs targeting your gene of interest
- Transfection reagent for optimized shRNA delivery (TurboFect for transfection kits or calcium phosphate for transduction kit)
- Positive and negative shRNA controls
- Trans-Lentiviral Packaging Mix (included in transduction kits only)
- Guaranteed* silencing

*When used with shRNA starter kits according to kit instructions.

**Related products**

- GIPZ Lentiviral shRNA → See p. 35
- TRIPZ Inducible Lentiviral shRNA → See p. 36
- Trans-Lentiviral shRNA Packaging Kit → See p. 63
- GIPZ and TRIPZ shRNA Controls → See p. 57

**shRNA knockdown guarantee**

When you purchase a minimum of three GIPZ or TRIPZ shRNAs to the same target, at least one of the shRNA constructs will reduce target mRNA levels by 70% or more when used with shRNA Starter Kits protocols and normalized for delivery efficiency. Transfection conditions should be confirmed using appropriate positive shRNA controls provided in the kit, and the percent knockdown should be compared to cells delivered with the non-targeting shRNA.

**High-titer lentiviral particle production**

Save time and money by transducing your cells of interest with high-titer lentiviral particles.

Lentiviral particle manufacturing eliminates the substantial investment of time, labor and money required in the design, production and quality control of functional shRNA reagents for transduction. All you have to do is order your gene of interest and then add viral particles to cells.

**SMARTchoice shRNA** (p. 35), GIPZ shRNA (p. 42) and Precision LentiORF clones (p. 87) are available as lentiviral particles.

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5' ![Clone oligonucleotides into a highly functional scaffold](image)

Sequence multiple clones to identify constructs with correct sequence

Transfect into packaging cells with helper plasmids encoding Gag/Pol and appropriate envelope proteins

Isolate viral supernatant and concentrate

Transduce into cells of interest

Assess gene silencing at mRNA and/or protein level

**All you have to do**

SMARTchoice shRNA (p. 35), GIPZ shRNA (p. 42) and Precision LentiORF clones (p. 87) are available as lentiviral particles.