RNA Interference with Guaranteed Knockdown!

shRNA, siRNA & microRNA

shRNA & siRNA mediated Knockdown
microRNA Detection with SYBR-Green qPCR Experiments
microRNA Expression
microRNA Target Validation with 3′-UTR Clones

www.origene.com
Dicer-Substrate Technology for shRNA & siRNA

27mer-29mer delivering higher knockdown than traditional 21mer

By its optimal length, HuSH-29 shRNA & Trilencer-27 siRNA have the advantages of improved efficacy and minimal interferon response. The length and design of OriGene’s RNAi substrates are important improvements over the use of traditional 21mer designs. Longer shRNA & siRNA constructs appear to enter the RNAi pathway more efficiently and result in much higher potency and specificity than shorter RNAi forms. However, in most mammalian cells, long double-stranded RNA provokes an interferon response as part of an antiviral defense. To overcome this obstacle, OriGene designs shRNAs & siRNA of less than 30 base pairs in length, which evade the mammalian immune system while still initiating strong and specific gene silencing.

A comparative study of different siRNA designs was conducted in a Nature Publication (Reference 1). According to the publication, “short RNAs that are long enough to serve as Dicer substrates (D-siRNA) can often evoke more potent RNA interference than the corresponding 21-nt siRNAs; this is probably a consequence of the physical handoff of the Dicer-produced siRNAs to the RNA-induced silencing complex.”

Comparison of gene knockdown using dicer-substrate siRNA and 21 mer siRNA

Key publications on dicer-substrate technology

2. Principles of Dicer Substrate (D-siRNA) Design and Function, Methods in Molecular Biology, 442: 3-10

Read more about our siRNA here: www.origene.com/sirna
HuSH-29 shRNA — Guaranteed Gene Knockdown

Comprehensive coverage of human, mouse & rat genes

Features & Benefits
- **29mer shRNA**: higher potency & minimal interferon response
- **Lentiviral particles available**: transduce almost all cells
- **Four retroviral vectors**: including GFP & RFP for transfection monitoring and multiple selection markers to carry out double knockdown experiments
- **Transient/stable transfection or retroviral infection**
- **shRNA kit**: 4 gene-specific constructs + scrambled control

Double Knockdown Experiment

**Scrambled shRNA (GFP Vector) + Scrambled shRNA (RFP Vector)**

**shRNA against RFP (GFP Vector) + shRNA against GFP (RFP Vector)**

Schematic diagram of 2 available GFP vectors
Both vectors have been validated through publications.
Cat# TR30007 (left) and Cat# TR30023 (right)

Exact-shRNA: Custom shRNA Design Service
Design any shRNA or miRNA with our synthesis service

The same superior design that is available for our pre-designed HuSH-29 sets is also offered through our Exact-shRNA service.
- Self-design or let OriGene design it for you
- Target species other than human, mouse or rat
- Integrate an effective siRNA sequence into an shRNA vector
- Reproduce the result of a published shRNA sequence
**HuSH-29 shRNA & Trilener-27 siRNA**

### In vitro assessment of shRNA targeting HIF1A

**4 HIF1A HuSH Vectors in HuSH TR320380 Kit**
- Control pRS Vector
- T137373
- T1373738
- T1373879
- non-effective shGFP

**HIF1A Blot: Anti-HIF1A**

**Erk1**
**Erk2**
**ReBlot: Anti-ERK1/2**

**Downregulation of HIF1A Expression by HuSH Constructs**

### In vivo effects of shRNA targeting Sprouty4

(Cat# TR509780) PLoS ONE. 2009; 4(5): e5467

**WT**
**Spry 4 KO**

**Spry2/Spry4 shRNA**

### Key publications


Learn more about our shRNA at www.origene.com/shrna

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**Trilencer-27 siRNA**

**Guaranteed gene silencing for human genes**

Similar to HuSH-29 shRNA, OriGene’s Trilencer-27 siRNA utilizes a 27mer Dicer-Substrate design that as the advantages over traditional 21mer of improved efficacy and minimal interferon response.

### Features & Benefits

- **Genomewide coverage** against human, mouse and rat
- **Higher potency & minimal interferon response**
- **siRNA kit:** 3 gene-specific siRNAs + 1 negative control

### siTRAN 1.0 siRNA transfection reagent

- Dual purpose reagent—transfect both siRNA duplex and corresponding cDNA clone
- High transfection efficiency and low cytotoxicity
- Cat # TT300001, TT300002 & TT300003
**microRNA Expression Plasmids**

**Comprehensive coverage for human, mouse, and rat genomes**

OriGene provides clones for over-expression of the microRNA (miRNA) of your choice. OriGene's miRNA precursor contains pre-miRNA (60-70nt) with 250-300 nts up- and down-stream of the flanking sequence. It is amplified from human genomic DNA and cloned into OriGene's pCMV6-Mir Vector. Upon transfection, the cellular machinery will process the CMV-driven expression of miRNA precursor into mature miRNA and cellular function can be analyzed.

**Features & Benefits**
- Genome wide miRNA coverage — 1829 human, 1160 mouse, and 436 rat
- Sequence confirmation of the precursor miRNA
- GFP for transfection monitoring
- Neomycin selection for stable cell establishment

GFP transfection of microRNA expression plasmids in HEK293 cells

Read more about miRNA at www.origene.com/microRNA

**GFP transfection of miRNA expression plasmids in HEK293 cells**

<table>
<thead>
<tr>
<th>Mir205</th>
<th>Mir143</th>
<th>Mir34b</th>
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<tbody>
<tr>
<td>Empty Vector</td>
<td>Non-transfected</td>
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**miRNA expression plasmids**

Sold individually as 10ug transfection-ready DNA or can be purchased as following sets

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>SC410001</td>
<td>Mouse miRNA expression plasmid set (486 vectors, 10ug each in 2-D bar coded tubes)</td>
</tr>
<tr>
<td>SC420001</td>
<td>Human miRNA expression plasmid set (652 vectors, 10ug each in 2-D bar coded tubes)</td>
</tr>
<tr>
<td>SC410002</td>
<td>Mouse miRNA expression plasmid set (486 vectors, 2ug each in 96-well plates)</td>
</tr>
<tr>
<td>SC420002</td>
<td>Human miRNA expression plasmid set (652 vectors, 2ug each in 96-well plates)</td>
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Luciferase reporter assays for the human genome

The 3' UTR plasmids provide a convenient solution for quantitative assessment of the inhibitory effect between miRNAs and their potential gene targets. OriGene’s 3' UTR clones were designed by cloning the 3' UTR sequence of a gene of interest, downstream of the firefly luciferase gene. The chimeric transcript level is then regulated by its interaction with miRNA(s), which results in varied luciferase activity quantifiable by a colorimetric assay.

Features & Benefits

- Genome wide coverage (>20,000 human genes)
- Firefly luciferase as the easy-to-assay reporter
- RFP for transfection monitoring
- High sensitivity from IRES-driven translation of the expression cassette

Find out more at www.origene.com/3-utr-clones

OriGene has used a new design adapted from C.P.Petersen et al. 2006, to dramatically increase the sensitivity of detection by decreasing the 3'UTR-luciferase reporter expression to a very low level.
qSTAR microRNA qPCR Detection Assays

Quantify your results down to the absolute copy number!

OriGene’s unique primer-based, SYBR Green qPCR miRNA detection system not only offers researchers a fast and simple method for profiling miRNA expression levels, but also provides means to quantify the results down to absolute copy number of miRNA.

Features & Benefits
- Genome wide coverage of human and mouse miRNA
- Determine absolute copy number of your miRNA with template standards
- Detect miRNA directly from total RNA samples

Products offered in qPCR miRNA detection system

<table>
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<th><strong>1</strong></th>
<th><strong>2</strong></th>
<th><strong>3</strong></th>
<th><strong>4</strong></th>
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<tr>
<td>Total RNA Purification Kit</td>
<td>First Strand cDNA Synthesis Kit</td>
<td>miRNA Primer Pairs or miRNA Primer Panels</td>
<td>miRNA Template Standards</td>
<td>SYBR Green Master Mix</td>
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</tbody>
</table>

*Components 2, 3 & 4 are unique and should only be used alongside OriGene’s qPCR miRNA detection system

First-strand cDNA Synthesis Kit
Two-step protocol
- Addition of poly (A) tail to RNA sample
- Use of anchor linker oligo dT to synthesize first-strand cDNA
- Cat# NP100041 & NP100042

miRNA Copy Number Standards
- Unique offering only from OriGene
- Determine the absolute transcript copy number of an experiment sample using the standard curve method

Find more information at www.origene.com/qpcr-mirna
OriGene, Your Partner in Research, Diagnostics and Beyond

- cDNA Clones/Lenti & AAV Particles
- CRISPR/Cas9/sgRNA
- Expression Vectors
- Recombinant Proteins
- Antibodies
- RNAi
- Normal & Cancer Tissues