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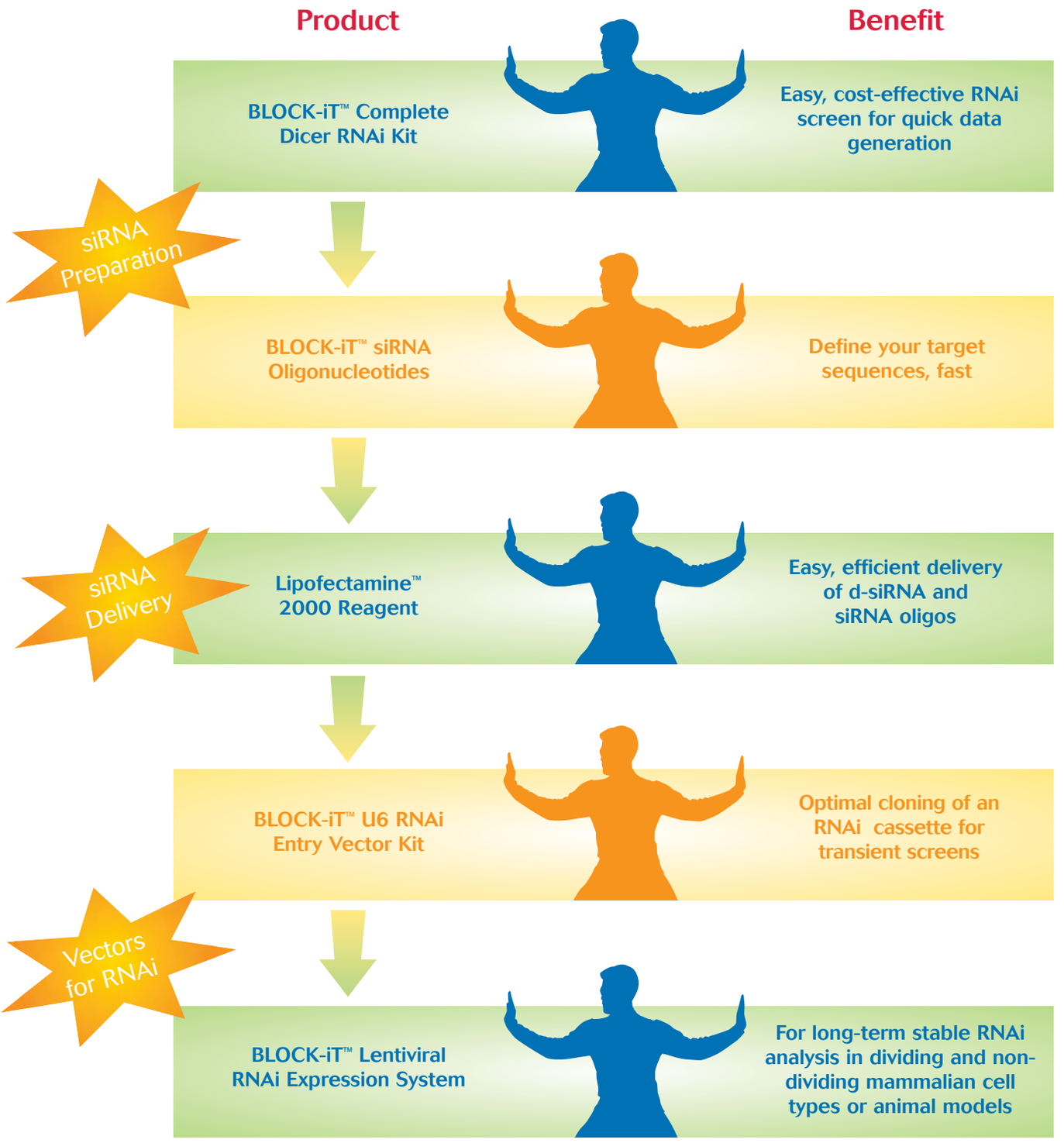
BLOCK-iT™ Products: Powerful tools to advance RNAi analysis

With BLOCK-iT™ RNAi Technology you can:

- Get powerful blocking and easily generate functional RNAi data
- Effectively prepare and deliver short interfering RNA (siRNA)
- Efficiently deliver and complete RNAi analysis in the broadest range of cell types

BLOCK-iT™ Tools for RNAi Analysis

Only BLOCK-iT™ Products give you everything you need for RNAi research



For more information on specific applications see page 3.

Powerful BLOCK-iT™ RNAi Technology

BLOCK-iT™ RNAi Technologies give you access to a wide range of RNAi applications. You'll easily generate functional data, effectively prepare and deliver siRNA, and efficiently complete RNAi analysis in any organism, mammalian cell type or animal model.

Harness the power of RNAi

RNA interference (RNAi) is rapidly becoming the most preferred technique for blocking the expression of a specific gene. RNAi technology allows scientists to turn off the genes they want to study. This provides an exciting new opportunity to examine the cellular phenotype in the absence of gene expression, and to assign gene function. Initially RNAi was shown to be a powerful method to decrease the expression of specific genes by the introduction of long double-stranded RNA (dsRNA) in invertebrates such as *Drosophila* and *C. elegans* (reviewed in 1,2). Today, modified RNAi knockdown techniques are available for analysis in eukaryotic cells. BLOCK-iT™ RNAi Technologies allow you to harness the power of RNAi—and take advantage of versatile techniques—in your gene knockdown studies.

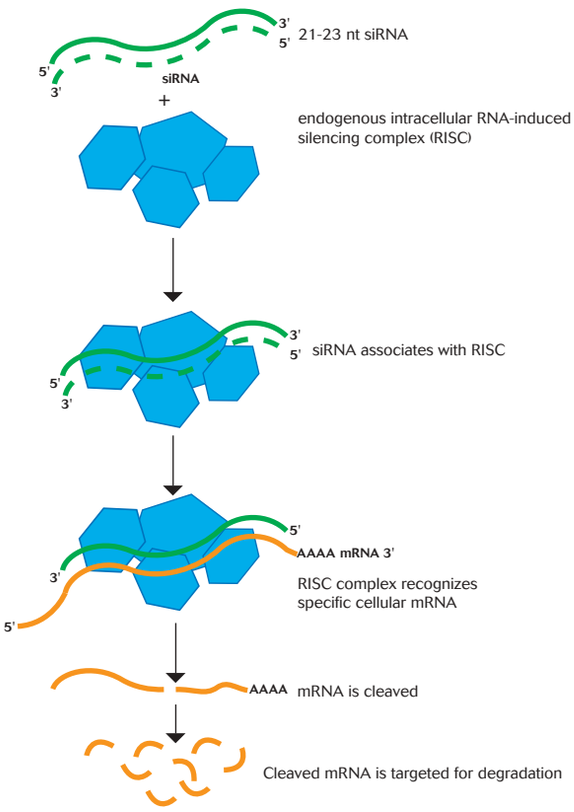
The endogenous RNAi mechanism

The RNAi process occurs endogenously in eukaryotes by the cleavage of long dsRNA into 21-23 nucleotide short interfering RNA (siRNA), facilitated by an enzyme called Dicer. The siRNA become part of an intracellular RNA-induced silencing complex (RISC) that targets complementary cellular mRNA for degradation (Figure 1). Ultimately, gene expression is blocked in the cell. BLOCK-iT™ RNAi Technologies provide several methods to generate siRNA in the cell:

- *In vitro* transcription and dicing of double-stranded RNA (dsRNA)
- Synthetic siRNA
- Vectors carrying an RNAi cassette

To find out more about which method is the best for you now and in your future RNAi applications, read on.

Figure 1 - Proposed mechanism for how RNAi works



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Meet all your RNAi research objectives

Get the level of repression you need for all your RNAi experiments. You may be at the beginning of your analysis, screening and characterizing several genes, wanting to use siRNA to determine the most effective target sequences, or studying long-term expression in primary mammalian cells. Whatever your experiment, there's a BLOCK-iT™ product that will work for you (Table 1).

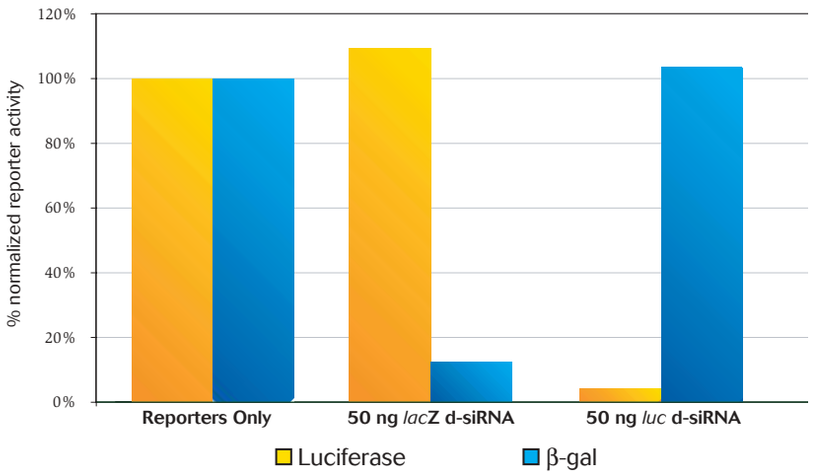
Table 1 - Find the BLOCK-iT™ Product that will work for you

Product	Application	Advantages
BLOCK-iT™ Dicer RNAi Transfection Kit	Analyze RNAi response in mammalian cells	• Fast results–Generate RNAi data right away • Effective blocking–A diced pool of siRNA sequences (d-siRNA) will likely contain a number of highly effective siRNA duplexes • Save time–No need to design and predetermine best target sequences • Cost-effective–Hundreds of experiments from one kit
Lipofectamine™ 2000	Most widely used reagent for siRNA delivery in mammalian cells	• Proven delivery–For successful gene knockdown • Deliver to a wide variety of cell types–Highly effective siRNA and d-siRNA delivery in most mammalian cells
BLOCK-iT™ RNAi TOPO® Transcription Kit	Generate long dsRNA for direct use in invertebrate RNAi analysis	• Efficient and easy–High-yield preparation of double stranded RNA (dsRNA) transcripts • Convenient-Start with any PCR product
BLOCK-iT™ Oligonucleotides for RNAi	ssRNA or siRNA Oligonucleotides	• Measureable–Identify the best target sequences for optimal specificity and desired level of blocking • Fast results–Get data right away • Easy–Simply design, order and transfect
BLOCK-iT™ U6 RNAi Entry Vector	Transient shRNA expression in most mammalian cells	• Fast, easy cloning of shRNA sequences–Convenient to screen multiple genes or sequences • Cost effective–Uses two ssDNA oligos • Easy transfer of U6 cassette to other vectors–*att* sites for efficient recombination
BLOCK-iT™ Lentiviral RNAi Expression System	Stable shRNA expression in dividing and non-dividing mammalian cells or animal models	• Effective long-term RNAi analysis–efficient lentiviral integration • Easy recombination of U6 cassette–*att* sites make transfer from the U6 entry vector fast and simple • Efficient lentiviral delivery–for reproducible results in animal models, hard-to-transfect, primary, and non-dividing cell types

Fast, easy RNAi screening using Dicer

The BLOCK-iT™ Complete Dicer RNAi Kit and BLOCK-iT™ Dicer RNAi Transfection Kit provide a rapid, easy way to screen up to five genes using RNAi in cell-based assays. You don't need to know the best siRNA target sequence for knockdown. Just begin with long dsRNA as a template for the BLOCK-iT™ Dicer reaction. Using an optimized protocol, you'll generate a pool of d-siRNA from the long dsRNA by simply adding the high-activity Dicer enzyme. Next, an easy spin column purification results in a highly pure pool of d-siRNA ready for efficient delivery to cells with Lipofectamine™ 2000 Reagent. Ultimately, you'll get high levels of specific gene blocking using the BLOCK-iT™ Dicer Kits (Figure 2).

Figure 2 - Get > 80% targeted gene knockdown with the BLOCK-iT™ Complete Dicer RNAi Kit



Diced pools of siRNA (d-siRNA) generated from β-galactosidase or luciferase dsRNA transcripts were used to determine the effectiveness of d-siRNA to specifically knock down gene activity. GripTite™ 293 MSR cells were co-transfected with pcDNA™1.2/V5-GW/*lac*Z positive control plasmid and pcDNA™5/FRT/*luc* expressing β-gal and luciferase reporters respectively, alone, or with 50 ng of *luc* or *lac*Z d-siRNA. For each reporter, the respective purified d-siRNA effectively reduced the expression of reporter activity, while d-siRNA derived from the other transcript did not.

Advantages of BLOCK-iT™ Dicing Technology

You can feel confident in your results since a number of highly effective siRNA are represented within each pool of diced transcript. In addition, the BLOCK-iT™ Dicing method for preparing siRNA has several advantages over other methods currently used for rapid screening of genes using RNA interference. With the BLOCK-iT™ Dicer RNAi Kits you will:

- Save time by going right to the screening phase, avoiding extensive siRNA design steps
- Get data right away by eliminating unnecessary analysis to identify mRNA target site availability or specificity
- Assure efficient blocking by using a mixture of d-siRNA

With these advantages you'll get valuable results in a variety of cell types even if allelic variation of the gene is present. For the initial generation of siRNA data in your system, the BLOCK-iT™ Dicer RNAi Kits are your best option.

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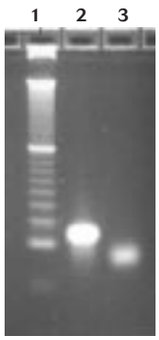
More efficient siRNA generation

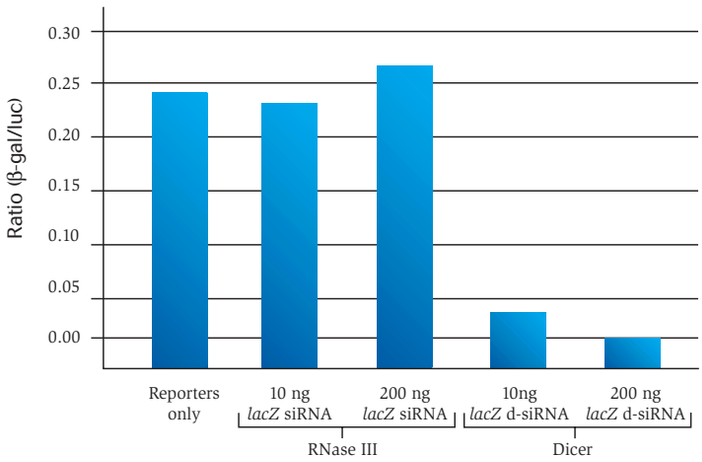
The high-quality BLOCK-iT™ Dicer Enzyme efficiently generates 21-23 nucleotide siRNA duplexes. These diced products are effective in specifically blocking gene expression. Other RNase III enzymes often produce smaller products (Figure 3). These small RNA may not be effective for an RNAi response, even at high concentrations (Figure 4). Take advantage of the BLOCK-iT™ Dicer method and effectively block gene expression. With BLOCK-iT™ Dicer RNAi Kits you'll get the results right away, without resorting to high concentrations of small RNA fragments prepared with other RNase III enzymes that may not give you the blocking you need.

Figure 3 - Diced dsRNA results in 21-23 nt d-siRNA while RNase III cleavage does not



d-siRNA was generated using the BLOCK-iT™ Complete Dicer RNAi Kit and using a commercially available RNase III-based kit according to manufacturers' directions. The BLOCK-iT™ Dicer enzyme generates dsRNA 21-23 nt in size (lane 2), compared to 12-15 nt products generated by the RNase III enzyme (lane 3) as shown by comparison to a 100 bp ladder (lane 1) (4% E-Gel® Gel).

Figure 4 - siRNA generated using BLOCK-iT™ Dicer provides better results than a bacterial RNase III



GripTite™ 293 MSR cells were transfected with both a *lac*Z and luciferase control reporter, alone, or with 10 ng or 200 ng of the RNase III-generated siRNA or Dicer-generated d-siRNA shown in Figure 3. Luciferase and β-gal activity was determined 48 hours post-transfection with Lipofectamine™ 2000. The ratio of β-gal to luciferase activity is shown. Non-specific inhibition of luciferase expression was not observed.

Everything you need for effective RNAi results

The BLOCK-iT™ Complete Dicer RNAi Kit provides everything you need to obtain and transfect a pool of siRNA specific to your gene including:

- A high-quality preparation of Dicer enzyme that results in high yields of a 21-23 nucleotide d-siRNA pool effective to produce the desired RNAi effect
- An RNAi purification module specifically developed to extract pure d-siRNA from the dicing reaction
- The easy-to-use Lipofectamine™ 2000 Transfection Reagent with proven high-efficiency transfection in mammalian cells—essential for efficient gene knockdown
- A BLOCK-iT™ RNAi TOPO® Transcription Kit for rapid, high-yield synthesis of long dsRNA

With the BLOCK-iT™ Complete Dicer RNAi Kit, you can screen up to five genes and get fast reliable results. You'll move forward quickly to the next stage in your RNAi research.

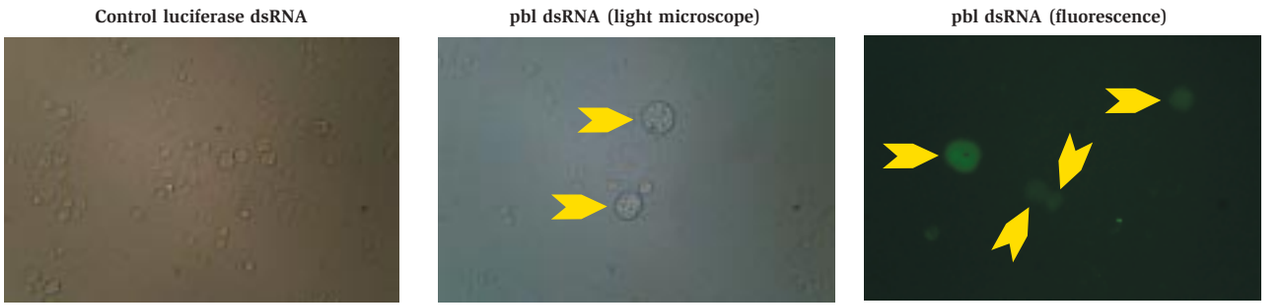
Rapid, high-yield RNAi TOPO® Transcription kit

If you are using nematodes or insect cells for RNAi analysis, the BLOCK-iT™ RNAi TOPO® Transcription Kit is ideal for you. You'll get high yields of pure long dsRNA that can be used directly to analyze the effects of gene blocking (Figure 5). Some mammalian stem cell types that do not exhibit an interferon response also show a response to transfected long dsRNA. With this kit you'll:

- Easily generate sense and antisense transcripts through the addition of BLOCK-iT™ T7-TOPO® linkers to any PCR product
- Get everything you need to synthesize, purify, and anneal RNA transcripts that can be used directly for RNAi analysis in invertebrates and some mammalian embryonic cells
- Reproducibly obtain a highly pure pool of d-siRNA when used in combination with the BLOCK-iT™ Dicer RNAi Transfection Kit

Use the BLOCK-iT™ RNAi TOPO® Transcription Kit and obtain high yields of the high-quality transcripts you need for RNAi analysis.

Figure 5 - BLOCK-iT™ generated transcripts result in clear RNA interference in insect S2 cells



To show suppression of endogenous gene function, RNAi analysis was performed using the gene for a *Drosophila* guanyl-nucleotide exchange factor *(pbl)* required for cytokinesis initiation. A unique knockdown phenotype is observed following the transfection of *pbl* long dsRNA. The BLOCK-iT™ RNAi TOPO® Transcription Kit was used to generate long dsRNA, and 0.5 µg of *pbl* dsRNA or a non-specific luciferase dsRNA were transfected using Cellfectin® Reagent. In the presence of *pbl* dsRNA, S2 cells of unusually large diameter are evident in a background of normally sized cells within 3 days of the transfection (right 2 panels). Cells of this size are only rarely seen in mock-transfected cells (not shown) or in those transfected with luciferase dsRNA (left panel), indicating that *pbl* dsRNA is inducing the phenotype.

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Highest quality siRNA or ssRNA

To successfully identify the best sequences for gene knockdown results, it is important that you begin with high-quality, error-free siRNA oligonucleotides. You'll get the high quality siRNA sequences essential to your experiments with both single and double-stranded BLOCK-iT™ RNA Oligonucleotides with an option for 50 or 200 nmole synthesis (Table 2). The custom BLOCK-iT™ siRNA Oligonucleotide service uses a unique Parallel Array Synthesizer (PAS) combined with a novel deprotection system and extensive monitoring of coupling efficiencies so you can rest assured that there is quality in every step of siRNA manufacturing. In addition you'll get:

- Quality Materials—All suppliers and raw materials rigorously validated prior to acceptance
- Quality process—PAS utilizes in-process QC so every custom siRNA oligo is monitored throughout the synthesis process
- Post synthesis quality check—QC by Mass Spectrometry to ensure the quality of each synthesis run
- Comprehensive certificate of analysis–So you can feel confident about the siRNA oligonucleotides you receive

With BLOCK-iT™ siRNA Oligonucleotide Service you can be certain that the experimental results you see are due to the sequence you ordered–not to oligo sequence errors or other contaminants.

Table 2 - Purification and Yields

Scale	Length	Desalted	HPLC
50 nmole	> 20 bases	5 OD	1 OD
50 nmole	< 20 bases	2 OD	.5 OD
200 nmole	> 20 bases	20 OD	3 OD
200 nmole	< 20 bases	8 OD	3 OD

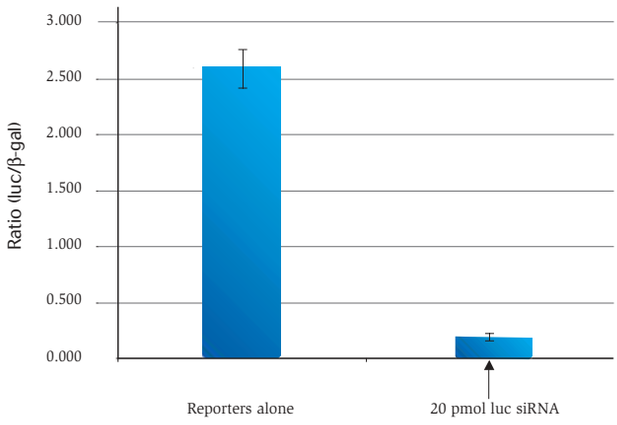
Superior results from PAS generated oligos

Custom BLOCK-iT™ siRNA Oligonucleotides are synthesized on a highly automated, computer controlled PAS for accurate synthesis. Invitrogen's unique PAS system allows for 96 Custom RNA oligos to be produced in parallel in industry standard 96-well format synthesis plates. The advanced "flow-through" of this process reduces the risk of contamination and handling errors, to assure that you'll get high-quality, accurate synthesis. The unique filtered-air drying system assures your siRNA oligos will remain stable in shipping. With the unique proprietary software and the PAS system used to prepare your siRNA oligos, you'll get:

- Reduced risk of nucleotide errors
- Efficient gene knockdown (Figure 6)
- Fast and easy oligo reconstitution

With BLOCK-iT™ siRNA Oligonucleotides you'll get the results you need by driving the quality of your oligos as high as it can go.

Figure 6 - BLOCK-iT™ siRNA Oligonucleotides specifically inhibit gene expression



GripTite™ 293 MSR Cells were transfected using Lipofectamine™ 2000 with two reporter constructs, one expressing luciferase and the other β-galactosidase, and with BLOCK-iT™ siRNA Oligonucleotides with a siRNA sequence specific to luciferase (luc siRNA). Luciferase and β-gal activity was measured in the absence and presence of the luc siRNA. While the luc siRNA had no effect on β-gal activity, the luciferase activity was significantly inhibited, and the ratio of luciferase to β-gal demonstrates the specificity and effectiveness of the siRNA to inhibit gene activity.

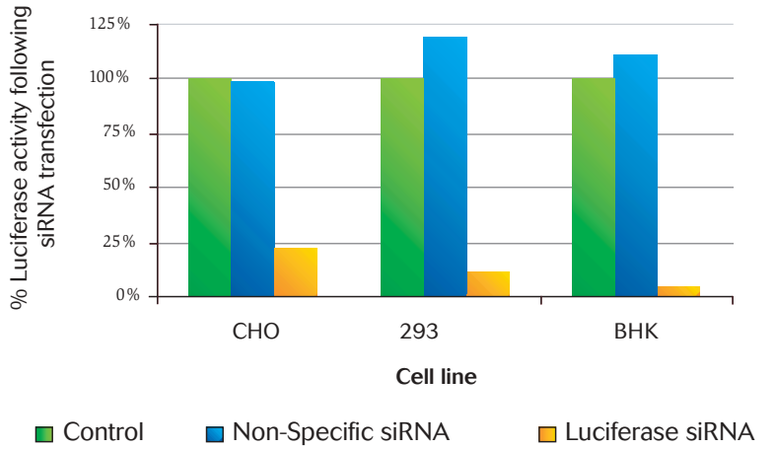
The most effective delivery of siRNA

Lipofectamine™ 2000 Reagent successfully delivers siRNA that leads to dramatic knockdown of gene expression in a wide variety of cells (Figure 7). Use Lipofectamine™ 2000 Reagent for your siRNA transfections and you'll get:

- High-efficiency transfection in mammalian cells for effective gene knockdown
- Easy-to-follow protocols
- Excellent performance on a wide variety of cell types

Let Lipofectamine™ 2000 be your key to successful siRNA delivery and gene knockdown. With Lipofectamine™ 2000, you—not your transfection reagent—choose the best cell line for your gene silencing studies.

Figure 7 - siRNA transfection with Lipofectamine™ 2000 leads to dramatic knockdown



CHO, 293, or BHK cells stably expressing luciferase were plated 24 hours prior to transfection. 20 pmol (293, BHK) or 10 pmol (CHO) of luciferase siRNA or non-specific siRNA was diluted in GIBCO™ Opti-MEM®, mixed with diluted Lipofectamine™ 2000 and added directly to the cells. Luciferase activity was assayed 24 hours following transfection.

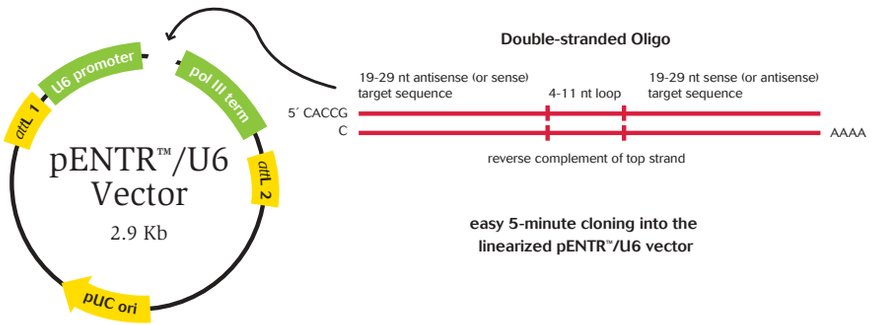
LUX™ Primers are ideal for RNAi research

LUX™ (Light Upon Extension) Fluorogenic Primers provide a sensitive, specific, and cost-effective way to confirm RNAi knockdown using real-time PCR. Each custom LUX™ Primer Set includes a primer labeled with a single fluorophore, and a corresponding unlabeled primer, both designed according to the specific sequence of your gene of interest. This primer set is all you need to perform PCR and the real-time detection of PCR products. There is no additional probe or quencher needed. You achieve superior real-time results cost-effectively. LUX™ Primers are the ideal way for you to confirm the effectiveness of your siRNA experiments.

Vectors for streamlined cloning of multiple target sequences

The BLOCK-iT™ U6 RNAi Entry Vector and BLOCK-iT™ Viral RNAi vectors provide a simple, streamlined approach to clone multiple short hairpin RNA (shRNA) target sequences for testing in transient transfections and subsequent expression using lentiviral or adenoviral delivery. A novel, simple cloning process is used to generate a vector that places a short DNA oligonucleotide immediately following a human U6 pol III promoter and upstream of a pol III terminator. This RNAi cassette will contain the sense and antisense regions complementary to the target gene of interest and "loop" nucleotides. This will then be transcribed into a shRNA molecule in the cell (Figure 8). The shRNA is processed into siRNA that acts to generate an RNAi effect. Once the cloning is complete, the BLOCK-iT™ U6 entry construct is ready to be used for initial screening in transient transfection experiments. If desired for powerful delivery to non-dividing and hard-to-transfect cell lines, primary cells, or animal models, you can easily transfer the U6 RNAi cassette into a BLOCK-iT™ RNAi lentiviral or adenoviral vector.

Figure 8 - Generate a mammalian expression vector with an RNAi cassette



dsDNA Oligonucleotides are annealed and incubated for 5 minutes at room temperature with the linearized pENTR™/U6 Vector. The mixture is transformed into One Shot® TOP10 Competent *E. coli*. The resulting plasmid DNA can be used immediately in a transient transfection. The shRNA expressed from the U6 promoter will form a hairpin that is processed into an siRNA molecule.

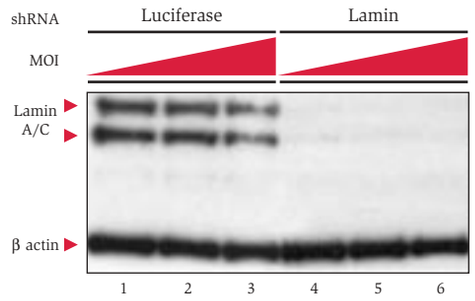
Efficient delivery and long-term shRNA expression
in any mammalian cell type

You'll get efficient, controlled delivery from the high-titer lentiviral stocks that can be prepared using the 293FT Cell Line and associated ViraPower™ reagents. Once you prepare a lentiviral stock, you can transduce your cells to see the RNAi response (Figure 9). The pLenti6/BLOCK-iT™-DEST Lentiviral RNAi vector provides you with the ability to observe long-term stable RNAi effects. This vector has all the required components for efficient packaging of the U6 RNAi cassette into virions so you can deliver shRNA to dividing, hard-to-transfect, and non-dividing cells (Figure 10). In addition the pLenti6/BLOCK-iT™-DEST vector contains:

- *att*R sites for efficient recombination of the U6 RNAi cassette from the U6 RNAi entry vector
- Viral delivery components and several safety features for safe, effective transduction
- Blasticidin selection marker for fast, efficient selection of cells stably expressing the shRNA

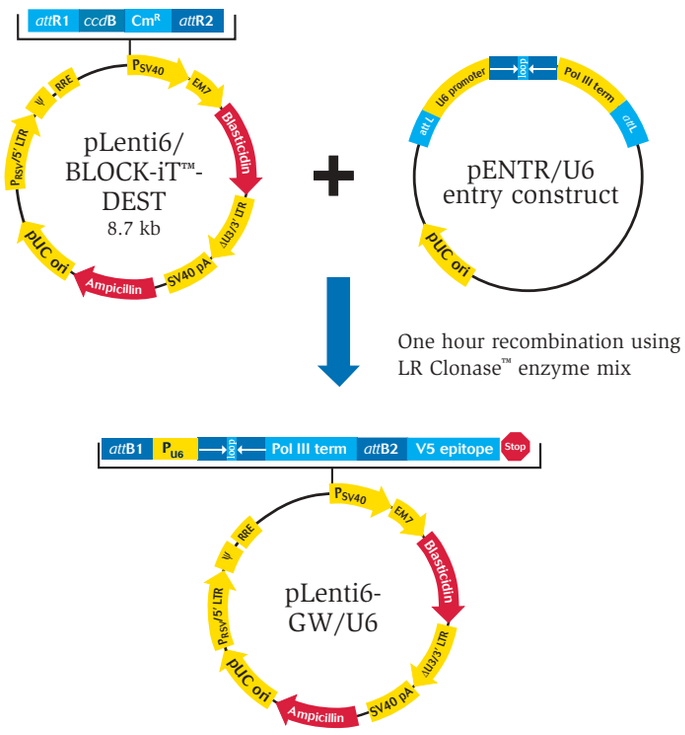
These features provide you with the flexibility to conduct transient or long-term stable RNAi experiments in hard-to-transfect, primary and other non-dividing mammalian cell types, and in animal models.

Figure 9 - Knockdown of endogenous gene expression using lentiviral shRNA delivery



HeLa cells were transduced with increasing MOI of pLenti6/BLOCK-iT™ virus encoding shRNA targeted to luciferase (control) or lamin A/C. Five days post-transduction, cell lysates were western blotted and probed with anti-lamin or anti-β-actin antibodies. Lamin-targeted shRNAs specifically inhibited lamin expression without affecting β-actin expression. The control luciferase shRNA had no effect on lamin expression.

Figure 10 - Using the pLenti6/BLOCK-iT™-DEST RNAi Vector



Glossary of terms

RNAi	RNA interference is a recently discovered functional tool. This is a phenomenon where an RNA introduced to a cell ultimately causes the degradation of the complementary cellular mRNA, and leads to the knock down of gene activity.
PTGS	Post transcriptional gene silencing; A phenomenon first identified in plants that has also been shown to occur in animals. Although PTGS was initially described as an endogenous method for viral defense and transposon silencing, it has now emerged as an exciting new research tool, RNA interference.
Cosuppression	Refers to the specific case of gene silencing in which RNA from a transgene and a homologous endogenous gene are suppressed at the same time.
Quelling	Cosuppression as described in *Neurospora crassa*–this term has only been used to describe silencing of a gene in fungi.
dsRNA	Double-stranded RNA; generally refers to long or full-length RNA duplexes. These large dsRNA initiate a general host cell shutdown in most mammalian cell types; the cells subsequently begin to decrease their expression of non-targeted genes and ultimately undergo apoptosis.
siRNA	Short (or small) interfering RNA; this is a short 21-23 nt RNA duplex involved in eliciting the RNAi response in mammalian cells.
RISC	RNA-induced silencing complex; this is the proposed complex made of multiple proteins that acts to bring the siRNA and the cellular mRNA together and activates a cleavage mechanism (likely endonucleolytic) so that the mRNA is released and degraded.
shRNA	Short hairpin RNA; also short interfering hairpin; *in vivo* can decrease the expression of a gene with complementary sequences by RNAi.
Pol III promoter	A pol III promoter, U6 or H1, is typically used to drive the production of shRNA. Pol III promoters have all the elements required for the initiation of transcription upstream of a defined start site.
Pol III terminator	Four or more T's for Pol III-dependent transcription termination.
U6 promoter	A pol III-type promoter that allows the production of shRNA with a defined end (see also Pol III promoter).
entry vector	A Gateway® vector containing *att*L sites used for cloning DNA fragments. The U6 Entry Vector, pENTR™/U6, is specifically designed with a U6 pol III-type promoter and terminator sequences such that RNAi target sequences can be cloned in which will form an shRNA in mammalian cells.
destination vector	Gateway®-adapted destination (DEST) vectors which contain *att*R sites and allow recombination with entry clones to form an expression vector. The BLOCK-iT™ RNAi Lentiviral and Adenoviral DEST vectors accept the RNAi cassette from the U6 Entry Vector. There are DEST vectors also available for the expression of proteins in prokaryotic, yeast, insect, or mammalian systems as native or fusion proteins.
LR Clonase™	An enzyme mix that facilitates recombination between *att*L and *att*R sites in an LxR Gateway® recombination reaction, to allow transfer of DNA sequences from an entry vector to a destination vector.

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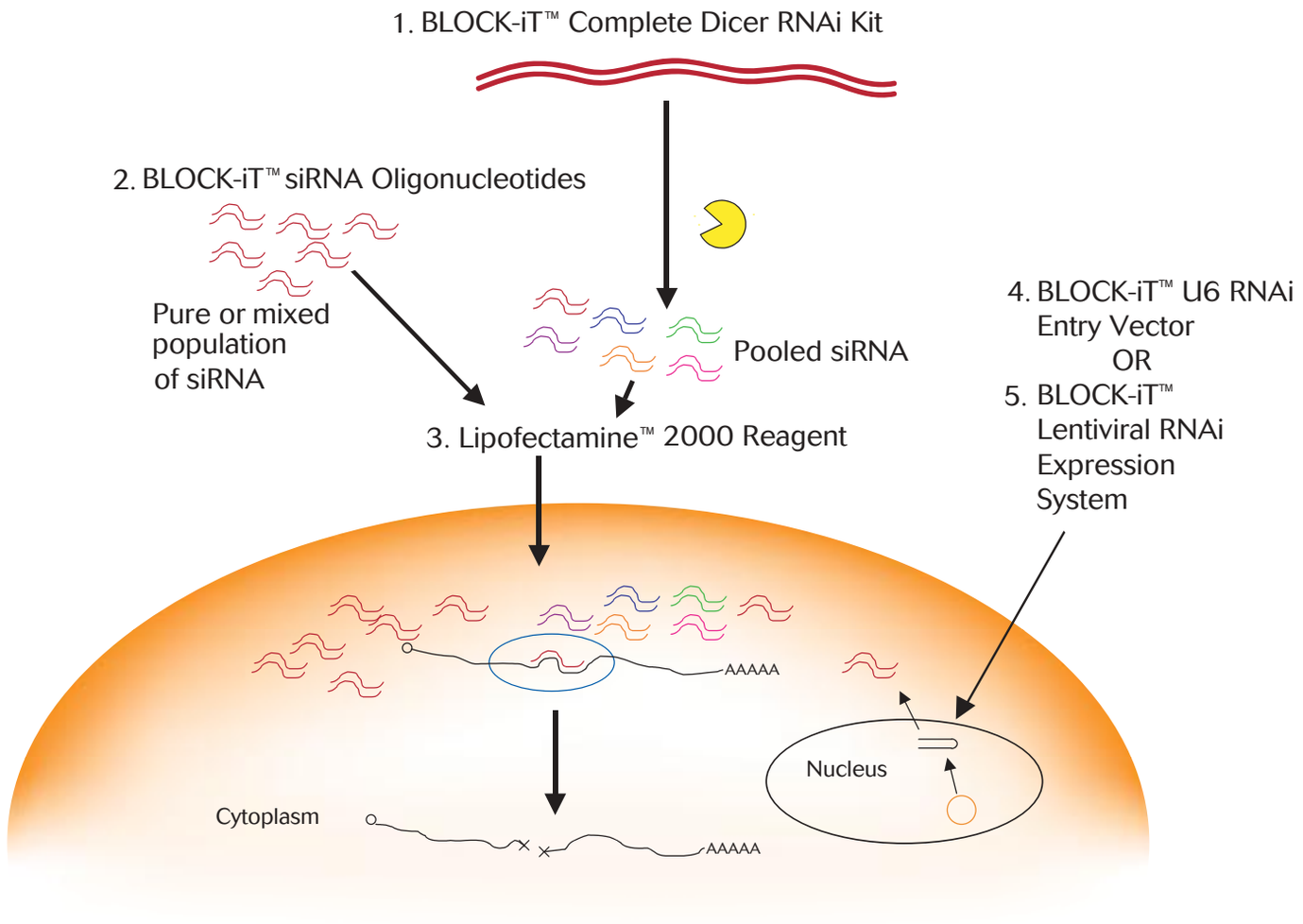
BLOCK-iT™ Products for powerful RNAi analysis



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Lipofectamine™ 2000 Reagent	0.75 ml	11668-027
	1.5 ml	11668-019
BLOCK-iT™ siRNA Oligonucleotides∝	Optional	Multiple
BLOCK-iT™ U6 RNAi Entry Vector Kit	20 reactions	K4945-00
BLOCK-iT™ Lentiviral RNAi Expression System	20 reactions	K4944-00
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* The BLOCK-iT™ Complete Dicer RNAi Kit contains the BLOCK-iT™ RNAi TOPO® Transcription Kit and the BLOCK-iT™ Dicer RNAi Transfection Kit.
† Based on transfections in 24-well plates
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