ON-TARGETplus™ siRNA

Specific
Up to 90% reduction of off-target effects with premium siRNA

Functional
Achieve guaranteed silencing with highly potent siRNA

Efficient
Save time and increase confidence in RNAi results

The New Standard for Specific Silencing
Dharmacon has expanded the market-leading siGENOME™ collection to directly tackle today’s greatest concern with RNAi – off-target effects.

ON-TARGETplus™ is the next generation of pre-designed siRNA reagents

Dharmacon has expanded the market-leading siGENOME™ collection to directly tackle today’s greatest concern with RNAi – off-target effects.

**Figures 1 and 2.**
Off-target effects and target mRNA silencing by unmodified and ON-TARGETplus siRNA were quantified using microarray and branched DNA analysis, respectively. Figure 1 represents off-target genes down-regulated by two-fold or more. Target mRNA knockdown is shown in Figure 2.

**ON-TARGETplus siRNA Reduces Off-targets While Maintaining Potency**

- Up to 90% reduction in off-target effects
- Guaranteed potent and specific silencing
- Achieve greater accuracy for increased confidence in gene silencing results
Off-Target Effects Blur True Results – Regain Clarity with ON-TARGETplus siRNA

Inconsistent phenotypes occur when siRNA silences unintended targets. These effects may be caused by either siRNA strand, and are independent of potent knockdown (Figure 3).

Target knockdown is not the only measure of an effective siRNA. Off-target effects can overshadow target silencing with false phenotypes.

Causes of Off-Target Effects
• Unintended knockdown by sense and antisense strand

Consequences of Off-Target Effects
• Inconsistent phenotypes leading to false positive and false negative results
• siRNA-induced cellular toxicity

Solution: ON-TARGETplus siRNA
• Groundbreaking bioinformatics enhance selection of highly specific siRNA
• Unique, patent-pending modification pattern improves specificity by reducing off-targets
• Pooled siRNAs increase target specificity

ON-TARGETplus siRNA Eliminates False Phenotypes and Maintains High Potency

Figure 3. False phenotypes are independent of target gene knockdown. The effect of silencing ARPC1B on cell migration was studied in a breast cancer cell line. A monolayer of cells was uniformly scraped and the rate of cell migration to close the scrape (wound healing) was evaluated. Both unmodified and ON-TARGETplus siRNAs induced potent target knockdown. Inconsistent phenotypes, due to off-target effects (red outline), were observed for cells transfected with unmodified individual siRNAs. ON-TARGETplus siRNAs reduced off-target effects producing a consistent phenotype.

In collaboration with Kaylene Simpson, Laura Selfors and Joan Brugge, Harvard Medical School

ON-TARGET\text{\textcopyright} plus siRNA is built on the foundation of the SMART\textsuperscript{TM} selection algorithm, the world’s most trusted rational siRNA design strategy.

In addition, ON-TARGET\text{\textcopyright} plus siRNA utilizes groundbreaking research from Dharmacon which demonstrates that matches between an siRNA seed region (positions 2-7 of antisense strand) and an mRNA 3’ untranslated region (UTR) are associated with off-target silencing (Figure 4).\textsuperscript{1} Incorporating these findings into ON-TARGET\text{\textcopyright} plus siRNA sets a new siRNA design standard that raises siRNA specificity to an unprecedented level.

**ON-TARGET\text{\textcopyright} plus siRNA minimizes seed region-related off-targets of the antisense strand**

- Designs optimized to favor antisense strand entry into RISC
- Design filter excludes siRNAs with known microRNA seed-region motifs
- Toxic effects from known stress-inducing motifs are eliminated from design
- Guaranteed target knockdown of 75% or better

\textbf{Figure 4.} Antisense strand seed regions contribute to off-target effects. A search for complementarity was performed between the siRNA antisense seed region (positions 2-7) and 5’ UTRs, ORFs, and 3’ UTRs of off-targeted and unaffected (not silenced) genes. The number of seed matches to the 3’ UTR region is almost five-fold greater in the off-targeted genes than the unaffected genes.

\textsuperscript{1} Birmingham, A., et al. “3’-UTR seed matches, but not overall identity, are associated with RNAi off-targets”, Nature Methods, 3.3 (2006): 199-204.
A recent publication demonstrates that off-target effects are primarily driven by antisense strand seed activity. Therefore sense strand inactivation alone does not decrease the total number of off-target genes. While sense strand inactivation promotes antisense strand loading into RISC, this can also result in more antisense-induced off-target events. ON-TARGETplus siRNA incorporates distinct modification patterns to both strands, thus significantly reducing the overall contribution of each strand to off-target events (Figure 5).

**ON-TARGETplus siRNA has the only modification pattern** that addresses off-target effects caused by both strands

- Sense strand is modified to prevent interaction with RISC and favor antisense strand uptake
- Antisense strand seed region is modified to minimize seed-related off-targeting

**The ON-TARGETplus modification pattern maintains siRNA potency while reducing off-target effects up to 90%**

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* Patent pending
Pooling of ON-TARGETplus siRNA Provides the Most Specific Silencing Available

Dharmacon SMARTpool® reagents combine four SMART selection-designed siRNAs into one highly effective siRNA pool. This combination of siRNAs mimics the natural silencing pathway, and is proven to have many advantages.

- Pooling reduces the effective concentration of individual siRNAs, a widely accepted strategy for reducing off-target effects (Figure 6)
- Save time and money with a single, highly functional siRNA reagent
- Reduce false negatives by targeting four different mRNA regions at once

The unequaled combination of superior bioinformatics, a unique chemical modification pattern, and the proven advantages of pooling results in virtual elimination of off-target effects.

Combining Pooling with the ON-TARGETplus Modification Consistently Produces Fewer Off-targets than Either Strategy Alone

Figure 6. Pooling of siRNAs reduces the overall number of off-targets. Off-target effects induced by the indicated siRNA reagents targeting 20 genes were quantified using microarray analysis. Data shown represents genes down-regulated by two-fold or more. Shaded box: Middle 50% of the data set. Line in box: Median value of the data set. Vertical bars: Minimum and maximum data values.
ON-TARGETplus Product Description Amounts Available

SMARTpool • A mixture of four siRNAs targeting one gene • Most recommended for potent gene knockdown • Guaranteed 75% target silencing 5, 10, 20, 50 nmol

Set of 4 Upgrade • Four individual siRNAs from corresponding SMARTpool reagent • Sequence information provided • Significant cost savings with Upgrade - concurrent or prior SMARTpool purchase required 4x2, 4x5, 4x10, 4x20 nmol

Set of 4 Individual Duplex • Up to four individual siRNA(s) from corresponding SMARTpool reagent • Sequence information provided 5, 10, 20, 50 nmol

siARRAY® Libraries • siRNA libraries grouped by pathway or gene ontology • Lower cost per gene than when purchased individually • Available as SMARTpool or individual siRNA reagents 0.5, 1, 2 nmol/reagent

Control Reagents • Validated positive and negative siRNA controls with reduced off-targets • Ideally suited for experiments using ON-TARGETplus siRNA reagents 5, 20 nmol

Scientific Leadership Drives Technological Advancement

Landmark peer-reviewed publications by Dharmacon scientists demonstrate a continued strong commitment to the scientific community. This pioneering spirit makes Dharmacon the global leader in RNAi technologies. ON-TARGETplus siRNA is the culmination of the most recent technological advancements:

• The ON-TARGETplus modification pattern reduces off-target effects while maintaining siRNA potency

• SMARTpool reagents reduce off-targets and enhance siRNA effectiveness

• Seed region analysis results in siRNAs with lower probability of off-target effects

ON-TARGETplus siRNA – the most specific and potent silencing reagent available


Related Dhharmacon Products of Interest:

Dharmacon is committed to developing new technologies in the field of RNAi. A partial list of the products offered by Dharmacon is listed below for your convenience. For a complete listing, and to order any of these products, visit www.dharmacon.com.

**DharmaFECT® Transfection Reagents**
- Consistently outperforms leading transfection reagents
- High levels of siRNA transfection
- Low cell toxicity
- Effective across a broad variety of cell lines

**siCONTROL® Reagents**
- Validated control siRNAs to ensure experimental reproducibility and silencing specificity
- Complete selection for multiple experimental applications
- Fluorescent controls for rapid estimation of transfection efficiency

**miRIDIAN™ microRNA Mimics, Inhibitors and negative controls**
- Highly potent oligonucleotides for modulating miRNA function
- Reliable reagents for reproducible studies of miRNAs in human, mouse and rat
- Advanced design for all human, mouse and rat miRNAs in latest miRBase database

Contact Information

For technical questions regarding the use of ON-TARGETplus products, please call Dharmacon Technical Support at (800) 235-9880 (toll-free, in the U.S.) or e-mail us at the addresses below:

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For more information or to order, visit: www.dharmacon.com