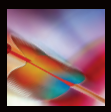
Thermo Scientific Dharmacon ON-TARGET*plus* siRNA
The Standard for siRNA Specificity

Reduce off-targets up to 90% with the market-leading siRNA



Combine higher target specificity with guaranteed silencing

Increase confidence in RNAi results with fewer false positives

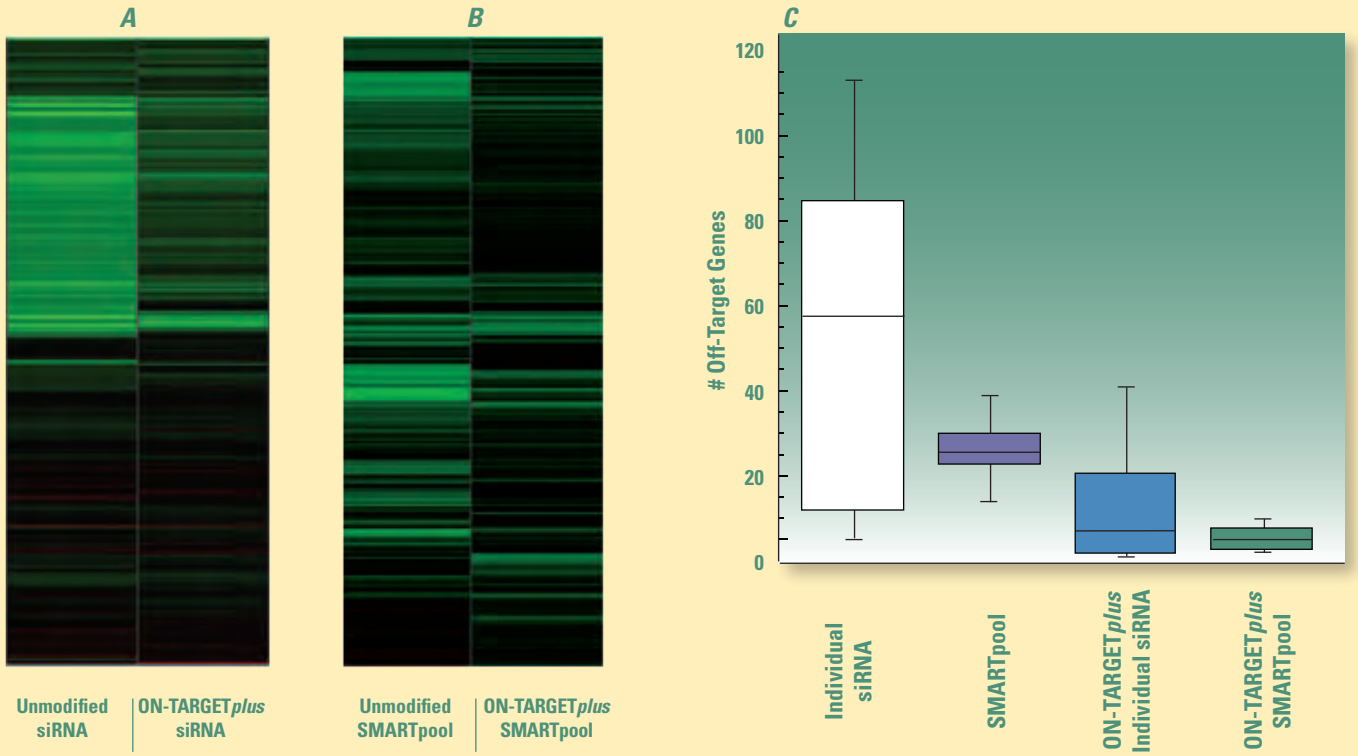
The standard for siRNA specificity

Trust Thermo Scientific Dharmacon ON-TARGET*plus*, the best-selling siRNA in the world, to provide the most complete, proven solution for reducing off-target effects that may lead to toxicity or false phenotypes.

- Unique dual-strand modification pattern to reduce off-targets caused by either strand
- Thermo Scientific Dharmacon SMARTpool technology to decrease off-targets and enhance siRNA effectiveness
- Seed region filters and seed frequency analysis for siRNA designs to minimize off-target effects

global market leader

ON-TARGETplus SMARTpool ™ reagents provide the most specific silencing available



ON-TARGETplus modifications reduce the overall number of off-targets, and pooling reduces them even further. *Off-targets induced by the indicated siRNA reagents targeting 5 genes were quantified by microarray analysis (Agilent 22K Platform).*

Panels A and B are representative examples of off-target signatures with and without application of ON-TARGETplus modifications to (A) a single siRNA and (B) a SMARTpool reagent. Panel C contains a boxplot of genes down-regulated by two-fold or more. Each box represents the middle 50% of the data set. Horizontal line in box: Median value of the data set. Vertical bars: minimum and maximum data values.

The only dual-strand siRNA modification available for unmatched reduction in off-targets

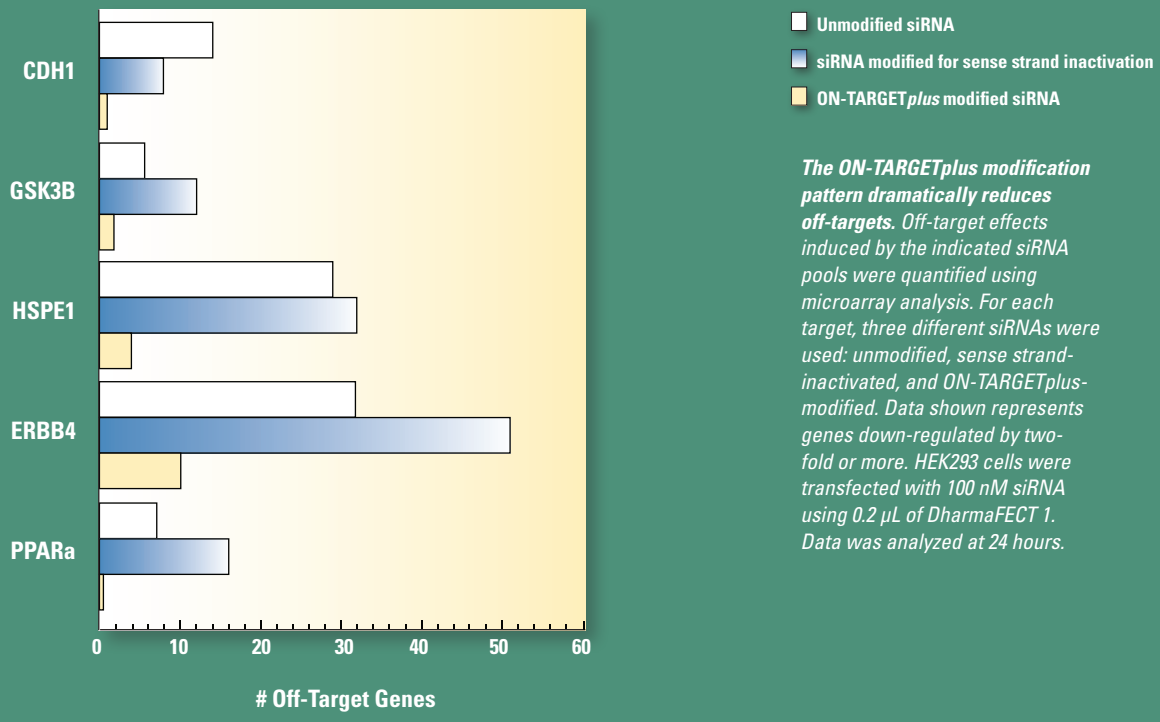
Studies have shown that both siRNA strands participate in the silencing of unintended targets[1]. Modifications that only promote antisense strand uptake may increase the number of off-targets caused by the antisense strand[2].

A two-strand mechanism requires a two-strand solution that can only be found with the patent-pending ON-TARGET*plus* modification pattern:

- Sense strand is modified to prevent uptake by RISC and favor antisense strand loading
- Antisense strand is modified to destabilize off-target activity and enhance target specificity

two-strand solution

ON-TARGETplus modifications reduce off-targets better than sense strand inactivation alone



The ON-TARGETplus modification pattern dramatically reduces off-targets. *Off-target effects induced by the indicated siRNA pools were quantified using microarray analysis. For each target, three different siRNAs were used: unmodified, sense strand-inactivated, and ON-TARGETplus-modified. Data shown represents genes down-regulated by two-fold or more. HEK293 cells were transfected with 100 nM siRNA using 0.2 µL of DharmaFECT 1. Data was analyzed at 24 hours.*

[1] Jackson, A.L., et al. "Position-specific Chemical Modification Increases Specificity of siRNA-mediated Gene Silencing." RNA 12.7(2006): 1197-1205.
[2] Chen, P.Y et al. "Strand-specific 5'0-methylation of siRNA duplexes controls guide strand selection and targeting specificity." RNA 2 (2008) 263-274.

Innovative bioinformatics advance ON-TARGET*plus* siRNA designs

ON-TARGET*plus* siRNA utilizes groundbreaking peer-reviewed research from Dharmacon, published in 2006, which was the first to demonstrate 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.[1]

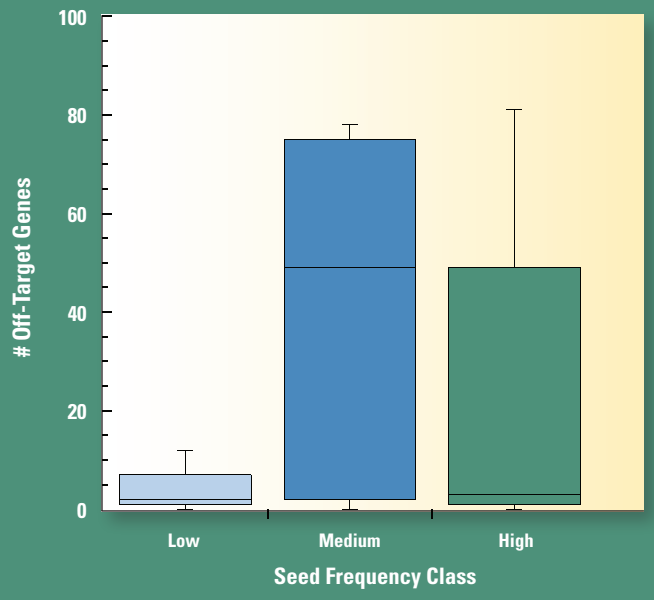
Additional findings in 2008 demonstrate that the frequency of a given seed in 3′UTR regions is an indicator of its likelihood of causing off-targets.[2]

Application of these findings to ON-TARGET*plus* siRNA designs set the standard for minimizing seed region-related off-targets of the antisense strand:

- Design filter excludes siRNAs with common microRNA seed-region motifs
- Known stress-inducing motifs are eliminated by design criteria
- Preference given to lower-frequency seed regions to minimize likelihood of miRNA-like off-target effects

groundbreaking research

siRNA designs with low-frequency seed regions ensure fewer off-targets [2]

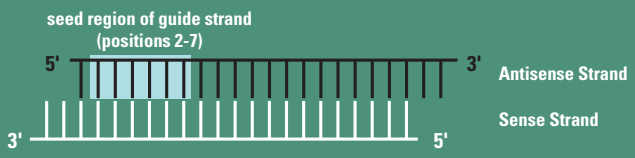


siRNAs with low seed frequency have a significantly lower number of off-targets than siRNAs with medium or high frequency seeds. *Five siRNAs with low, medium, or high frequency seed regions were transfected into HeLa cells and their associated off-target signatures assessed via global expression profiling (Agilent 22K platform).*

siRNA sequences were constant at positions 1 and 8-19, only the seed regions (positions 2-7) were altered (see below).

Low frequency seeds: <350 occurrences in the HeLa transcriptome.
Medium frequency: 2500-2800 occurrences.
High frequency: >3800 occurrences.

Figure adapted from Anderson et.al. See published citation for full experimental details.



[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.
[2] Anderson, et al.: "Experimental validation of the importance of seed frequency to siRNA specificity." RNA 14.5 (2008).

ON-TARGET*plus* SMARTpool reagents are proven to eliminate false phenotypes

SMARTpool technology combines four highly potent siRNAs to mimic the natural silencing pathway. Pooling reduces the concentration of each individual siRNA, a widely accepted strategy for reducing off-target effects.

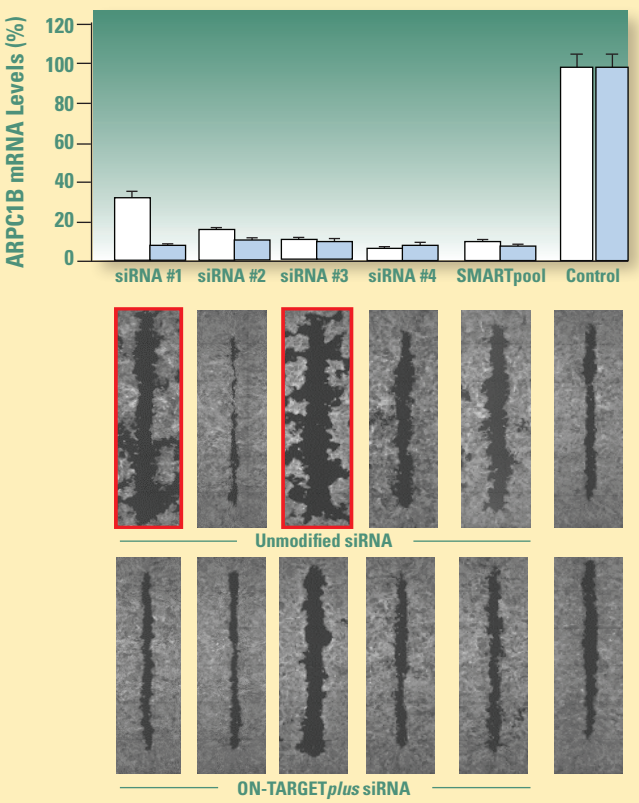
The unequaled combination of superior bioinformatics, a unique, dual-strand chemical modification pattern, and the proven advantages of pooling results in a dramatic reduction of off-target effects.

ON-TARGET*plus* combines these proven strategies into a single siRNA reagent, saving you time and money.

- Potent, guaranteed silencing with fewer off-targets that may confound true results
- Seed-region modifications and design optimization for the highest specificity available
- SMARTpool reagents reduce false negatives by targeting four different mRNA regions at once

superior bioinformatics

ON-TARGETplus siRNA eliminates false phenotypes and maintains high potency



Unmodified siRNA

ON-TARGET*plus* siRNA

False phenotypes are alleviated by ON-TARGETplus SMARTpool reagents while target gene knockdown is maintained. *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 siRNA reagents induced potent target knockdown. Inconsistent phenotypes due to off -target effects (red outline), were observed for cells transfected with unmodified individual siRNAs. The unmodified SMARTpool improved the false phenotype considerably, while the ON-TARGETplus SMARTpool significantly reduced off -target effects to produce a consistent phenotype.*

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

Trust your screening results to the leader in RNAi technologies

Only Dharmacon siRNA product lines provide a single source, comprehensive toolset for successful, complete siRNA screening.

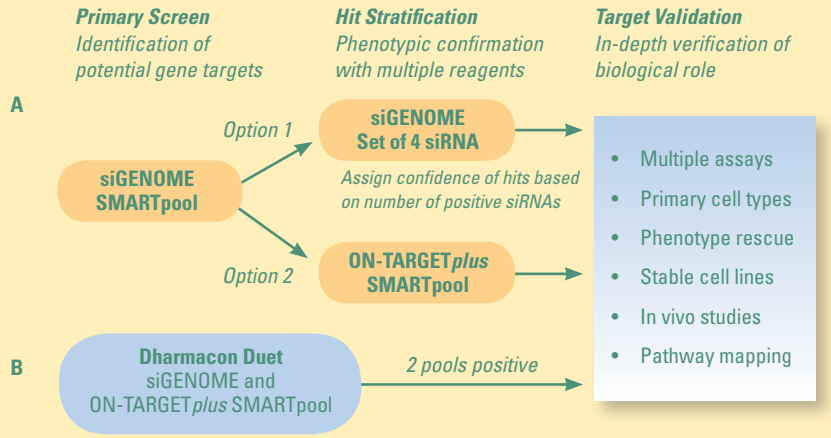
The practice of silencing a gene with multiple RNAi reagents is a standard technique for stratification of hits. ON-TARGET*plus* reagents are a key component for high-confidence hit stratification by reducing false phenotypes resulting from off-target effects.

No other provider has the breadth and scientific advantages of our siRNA products:

- More guaranteed reagents per gene for highly efficient target silencing
- More strategies for enhanced specificity to reduce false results
- More product formats to support multiple screening workflows

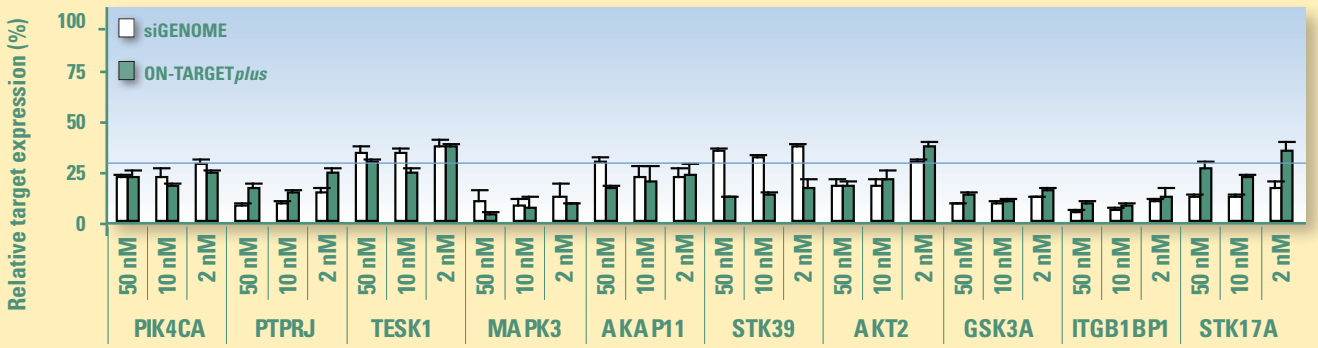
www.thermo.com/otp

High-confidence screens with Dharmacon siRNA reagents



Workflow diagrams for highly effective siRNA screen hit stratification. *(A) Two options for hit stratification following primary screen with siGENOME SMARTpool siRNA reagents. (B) An emerging method for selection of high-confidence hits from a primary screen - the Dharmacon Duet approach. Carrying out an initial screen with two SMARTpool siRNA reagents (siGENOME and ON-TARGETplus) reduces experimental time and effort to select high-confidence hits and allows researcher to rapidly proceed to in-depth target validation.*

ON-TARGETplus siRNA reduces off-targets while maintaining potency in RNAi screens



Target mRNA knockdown in a screen with siGENOME and ON-TARGETplus SMARTpool siRNA reagents was highly comparable, even at concentrations of 2 nM. In extended studies, ON-TARGETplus also was effective in reducing false phenotypes due to off-targets (manuscript in preparation).

Offering flexible formats to meet every experimental need

Ordering information and related products are below. For contact information please see the back cover of this brochure.

ON-TARGET*plus* Products	Description			Amounts Available
SMARTpool	A mixture of four predesigned siRNAs targeting one gene. Most recommended for potent gene knockdown. Guaranteed 75% target silencing. Sequence information provided.			5, 10, 20, 50 nmol
Set of 4 siRNAs or Individual siRNAs	Four individual siRNAs from corresponding SMARTpool reagent. Guaranteed 75% target silencing by 3 of 4 siRNAs. Sequence information provided.			4x2, 4x5, 4x10, 4x20 nmol 5, 10, 20, 50 nmol
Pre-defined siRNA Libraries	Available pre-defined siRNA Libraries with ON-TARGET*plus* siRNA reagents			0.5, 1, 2 nmol
	Apoptosis Cell Cycle Regulation Cytokine Receptors Deubiquinating Enzymes Druggable Genome	**Human Genome - NEW!** GPCR Ion Channels Kinases Membrane Trafficking Nuclear Receptors	Phosphatases Proteases Ubiquitin Conjugation - subset 1 Ubiquitin Conjugation - subset 2 Ubiquitin Conjugation - subset 3	
Custom siRNA Libraries	Custom human and mouse siRNA libraries available for your list of genes. Available as SMARTpool or set of four individual siRNA reagents per gene target.			0.5, 1, 2 nmol/ reagent
Custom Design and Synthesis	Our specialists will design and synthesize an optimal ON-TARGET*plus* SMARTpool for your particular needs (non-standard species, gene variant-specific silencing, etc.)			50 nmol
Custom siRNA Synthesis	Already have your siRNA sequence? Have it synthesized with ON-TARGET*plus* modifications to improve overall silencing specificity. In vivo processing and HPLC purification available.			20, 40, 100 nmol (>100 nmol, please inquire)
siRNA Control Reagents	Positive siRNA controls with reduced off-targets, validated to work in multiple cell lines. Negative controls are microarray confirmed to have nearly undetectable silencing in human cells.			5, 20 nmol

Thermo Scientific Dharmacon ON-TARGET*plus* siRNA

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