



siRNA SMARTpool®

Bcl2

Catalog # M-003307

Components

SMARTpool® Bcl2

Catalog # M-003307

Accession # NM_000633

One vial containing **5 nmoles** of 4 pooled SMARTselected siRNA duplexes with "UU" overhangs and a 5' phosphate on the antisense strand.

5X siRNA Buffer

Catalog # B-002000

One vial containing **1.5ml** of 100.0mM KCl, 30.0mM HEPES pH 7.5, 1.0mM MgCl₂.

Please read this product insert before use to obtain background information and a detailed protocol.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Shipping and Storage: The siRNA SMARTpool® is shipped at or below ambient temperature. Prior to hydration, the siRNA SMARTpool® and 5X siRNA Buffer may be stored at room temperature. After hydration of siRNA SMARTpool with 1X siRNA Buffer (or another appropriately buffered RNase free solution of your choice), aliquot as desired and store at -20°C.

Precautions: RNA oligos are susceptible to degradation by RNases which are present almost everywhere. They are also susceptible to non-specific degradation. For this reason, they should be handled and stored using RNase-free conditions and solutions. Gloves should be worn during handling and solutions should be treated to inhibit or destroy ribonucleases.

Technical Support

For questions or concerns regarding the use of the SMARTpools®, please contact Dharmacon Technical Support, 1-800-235-9880, email: lab@dharmacon.com.

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RNA Interference Overview

RNA interference (RNAi) is a highly conserved pathway used by eukaryotes as a cellular line of defense directed against invading viral genomes or as a method to clear a cell of aberrant transcription products^{1,2}. While the mechanism is not fully understood, RNAi is proving to be an invaluable tool for gene function analysis and target validation. Dharmacon's *SMARTselection*[™] and *SMARTpool*[®] technologies enable researchers to establish a successful RNAi program in their laboratories.

Cellular introduction of double-stranded RNA (dsRNA) induces RNAi in a diverse group of lower eukaryotic organisms³⁻¹¹. RNAi inhibits gene expression through sequence-specific, dsRNA-mediated degradation of the target messenger RNA (mRNA). Attempts to induce RNAi using long dsRNA in mammalian cell lines were first met with limited success, due in part to the induction of the interferon response, which results in a general inhibition of protein synthesis¹²⁻¹⁴.

In 2001, landmark studies revealed that short RNA duplexes (19-25 bases in length) introduced into mammalian cells in culture led to sequence-specific inhibition of target mRNA without inducing an interferon response^{15,16}. These short, or small interfering RNAs (siRNAs), act catalytically at non-toxic, sub-micromolar concentrations and are capable of cleaving up to 95% of the target mRNA in the cell. The siRNA-mediated effect has been shown to be stable over time, and silencing may be observed through several cell generations¹⁷. These properties make siRNA-mediated interference attractive as a method for targeted gene knockdown. The ability to assess gene function by this method represents an innovation that promises to revolutionize and accelerate genome-wide research, drug discovery, and therapeutic development.

For successful cell-based investigations, the keys to siRNA-dependent gene silencing depend on a number of critical factors:

1. Target sequence selection and siRNA design
2. Suitable cell line or cell culture system
3. Optimized delivery conditions (lipid mediated, electroporation, etc.)
4. Abundance and turnover rate of the target mRNA or protein of interest
5. Accuracy and ease of assaying for mRNA levels, protein levels or phenotype.

To assist researchers in addressing and minimizing potentially confounding issues associated with siRNA-dependent gene silencing, Dharmacon has developed an RNAi-specific technology platform.

SMART selection™ and SMARTpool® Platform Overview

The selection of functional siRNAs has been one of the major challenges for the successful application of RNAi. To address this, Dharmacon's R&D scientists set out to identify the sequence-specific and biophysical attributes of functional siRNAs. This was achieved by performing a systematic analysis of a large test set of siRNAs^{18,19} that led to the development of the SMARTselection™ and SMARTpool® technologies. SMARTselection™ strategies incorporate a multi-component algorithm that identifies siRNAs with a very high probability of potent and specific silencing. SMARTpool® reagents combine four SMARTselection™-designed siRNAs into a single pool, resulting in even greater probability that the SMARTpool® reagent will reduce target mRNA to low levels.

Dharmacon's SMARTselection™ and SMARTpool® technologies have been used to develop a truly viable genome-wide library of siRNA reagents. The SMARTselection™ and SMARTpool® platform has been validated in each of two common cell lines: HeLa and HEK 293. SMARTpool® reagents are expected to silence target gene expression at the mRNA level by at least 75% (F75) when used under optimized transfection and cell culture conditions. To date, Dharmacon's R&D group has found that over 85% of the SMARTpool® reagents reduce target mRNA levels by 95% (F95) or more. F75 and F95 are definitions for an siRNA that silences or reduces mRNA levels by 75% or 95%, respectively.

In addition to significantly improving the probability of potent silencing, the SMARTpool® reagent enables you to analyze a gene with only one assay and be guaranteed the siRNA reagent will function under appropriate cell culture conditions. This saves on assay cost, as well as time and uncertainty related to randomly designed siRNA duplexes. The performance guarantee when silencing is monitored at the mRNA level 24-48 hours after transfection with 100nM siRNA. Optimum transfection efficiency should be confirmed using one of Dharmacon's positive control siRNA duplexes or another previously validated functional siRNA duplex. Low transfection efficiency will result in lower levels of silencing. Transfection efficiency may vary between different cell lines, and should be optimized for each cell line used. The guarantee is backed by expert technical advice and involves careful evaluation of the context in which the siRNA was used (intended target, controls, transfection efficiency and general culture conditions, detection method and time points assessed, etc) in addition to the siRNA design.

Dharmacon SMARTpool® siRNAs are designed with the following features that have been shown to be optimal for effective silencing:

1. Twenty-one-nucleotide RNA oligonucleotides forming a 19-base-pair duplex core.
2. Symmetrical two nucleotide 3'-UU overhangs.
3. 5'-phosphorylated antisense strand.
4. Quality controlled duplex.
 - a. The average molecular weight of a duplex equals approximately 13,300g/mole.
 - b. Mass of sense and antisense strands is confirmed by MALDI-TOF mass spectrometry.
 - c. Duplex formation is confirmed by non-denaturing gel electrophoresis.
5. Duplexes are provided in the desalted form.
6. Complimentary 5X siRNA Buffer—validated and tested as a low salt buffer that is compatible with most tissue culture conditions.

Contents:

Item	Description	Quantity
SMARTpool® reagent	<ul style="list-style-type: none"> Four pooled SMARTselection™-designed siRNAs “UU” 3'-overhangs 5'-phosphate on the antisense strand 	5 nmoles
5X siRNA Buffer	100.0mM KCl 30.0mM HEPES-pH 7.5 1.0mM MgCl ₂	1.5ml

Attention! Product Change: 5X siRNA buffer is now provided with all SMARTpool® reagents. This replaces the 1X Universal buffer previously provided. When the 5X siRNA buffer is diluted with sterile RNase-free water to 1X, the components and final concentration of the 1X buffer remains the same (20mM KCl, 6.0mM HEPES-pH 7.5 and 0.2mM MgCl₂).

Other components required but not included as part of kit:

Reagents:

- Cell line
- Transfection Reagent
- Negative Control siRNA
- Reduced serum or serum-free media
- Serum-containing media
- 1 X Phosphate Buffered Saline (PBS)

Equipment:

- Standard cell culture capability

Basic siRNA Protocol (Lipid-mediated transfection for adherent cells)

1. Dilute the 5X siRNA Buffer to 1X by mixing 4 volumes of sterile RNase-free water with 1 volume of 5X siRNA Buffer.
2. The siRNA SMARTpool® reagent may be resuspended using 250µl of 1X siRNA buffer (or another appropriately buffered RNase free solution of your choice) for a recommended concentration of 20µM (20pmol/µl). This is sufficient for the following formats:

Format (wells/plate)	~Surface area (cm ²)	pmol per well	Final volume per well (ml)	siRNA final concentration (nM)	Number of wells per 1 nmole	Number of wells per 5 nmole
96	0.3	10	0.1	100	96	480
24	2.0	50	0.5	100	20	100
12	4.0	100	1.0	100	10	50
6	10	200	2.0	100	5	25

Final concentrations range from 1-200nM and should be optimized for the target of choice and assay conditions.

3. For lipid complex formation and subsequent transfection, we strongly recommend following the instructions provided by the transfection reagent manufacturer and taking measures to test and optimize the conditions best suited for the cell line or culture of choice. General recommendations include:
 - a. Cell density at ~70-90% confluent, or approximately 1 X 10⁵ cells/ml density, at the time of transfection (this will vary with the growth characteristics of the cells).
 - b. Standard incubation conditions for mammalian cells are 37°C in 5% CO₂.
4. The lipid encapsulated SMARTpool™ ready for transfection.

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Dharmacon is licensed to provide siRNA and RNAi products for biological and pharmaceutical research and development, excluding use in humans and use in clinical diagnostics. Dharmacon siRNA products may be used only by the purchaser and may not be resold without the express agreement of Dharmacon. The Massachusetts Institute of Technology granted one of four co-exclusive rights to Dharmacon to the claims in US Patent Applications 60/265232, 09/821832, and PCT/US01/10188, and non-US Patent Application European Serial Number 00126325. The Carnegie Institution of Washington granted rights to Dharmacon to the claims in US Patent Applications 60/068562, 09/215257, PCT/US98/27233.

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