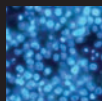
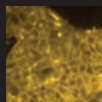




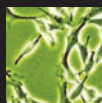
## Thermo Scientific Dharmacon Accell siRNA A new world of RNAi discovery



Effective siRNA delivery without a transfection reagent



Proven results in difficult-to-transfect cells



Specific and highly effective silencing



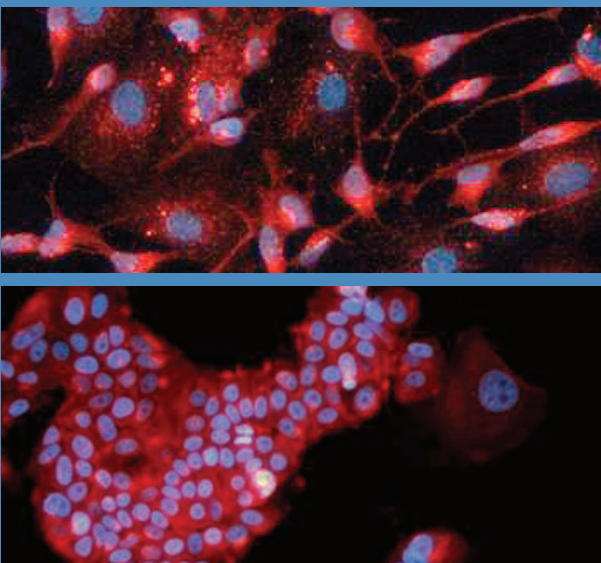
# siRNA delivery into difficult-to-transfect cells

RNAi research no longer is constrained by cell types that are resistant to conventional lipid-based delivery reagents. Thermo Scientific Accell siRNA provides delivery into difficult-to-transfect cell types for unprecedented experimental flexibility and discovery.

- Effective delivery with no transfection reagent or electroporation required
- Novel siRNA modifications for uptake, stability, and specificity
- Proven performance in neuronal, immunological and other difficult-to-transfect cell types

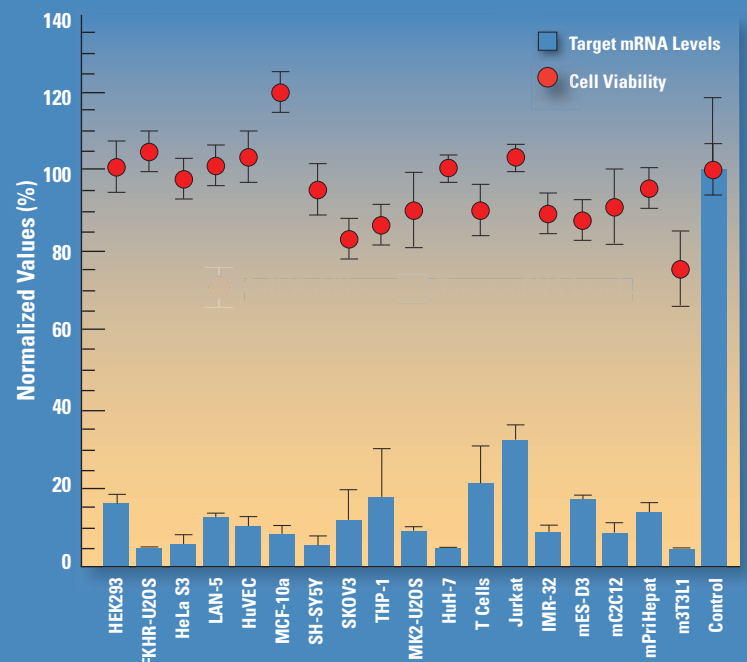
## siRNA modified for delivery

Efficient delivery of Accell® Red siRNA to SH-SY5Y and MCF-7 cells



Uptake of Accell siRNA confirmed by fluorescence microscopy. SH-SY5Y (top panel) and MCF-7 cells (lower panel) were treated with 1 µM Accell Red Cyclophilin B Control siRNA in Accell delivery media. (Red fluorescence = Cytoplasmic localization of Accell siRNA; Blue = Nuclear Hoechst dye).

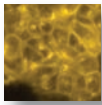
Accell siRNA provides reproducible, reliable results across cell lines



### Peer reviewed publications demonstrate effective silencing in numerous cell types

- Human Suspension Cells** BDCM, IM-9, Jurkat, K662, THP-1, Jeko-1 and Mino MCL
- Human Adherent Cell Lines** A-375, ARPE-19, CaCo-2, CG4, DLD-1, DU145, EC52, GTM-3, MR32, LAN5, LNCap, MIA PaCa-2, NCI/ADR-RES, OVCAR-3, OVCA-420, PGA1, PNT2, SH-SY5Y, SK-N-BE and SK-N-SH neuroblastoma, SK-BR3, SKOV3, TE15, U251, U87MG, U937
- Mouse and Rat Cell Lines** ES-D3, H9c2, LβT2, LNK, mouse oviduct epithelial cells, mProx24, PC-12
- Human Differentiated Stem Cells** Adipocytes\* and osteoblasts\*
- Human Primary Cells** HUVEC, HUASMC, hMSC, PBMC, astrocytes, hypothalamic neurons, T cells, NK cells, macrophages, immortalized B cells, esthesioneuroblastoma cells, hepatocytes, keratinocytes, bronchial smooth muscle cells
- Mouse and Rat Primary Cells** Cortical neurons, hippocampal neurons, striatal neurons, Naive CD4+ T cells, LN T-cells, D1 T cells, RAW 264.7 macrophages, hepatocytes, neonatal ventricular cardiomyocytes, derived fibroblasts\*\*, bone marrow-derived DC, NOD CD4+CD25- splenic cells, NOD BMMs, VSMCs, somatotroph GH3, McArdle 7777 cells (hepatoma cell line), osteoclast precursors, calvarial (cranial) organ, pituitary cell types\*\*\*

\*derived from hMSC, \*\*from genetically modified mice, \*\*\*derived from primary tumors



## Easy to use for rapid, high confidence results

Thermo Scientific Accell siRNA reagents are specially modified for uptake without a separate delivery reagent - no complexing wait time, no wash steps. Simply combine Accell siRNA with optimized Accell siRNA delivery media\* and add directly to cells.

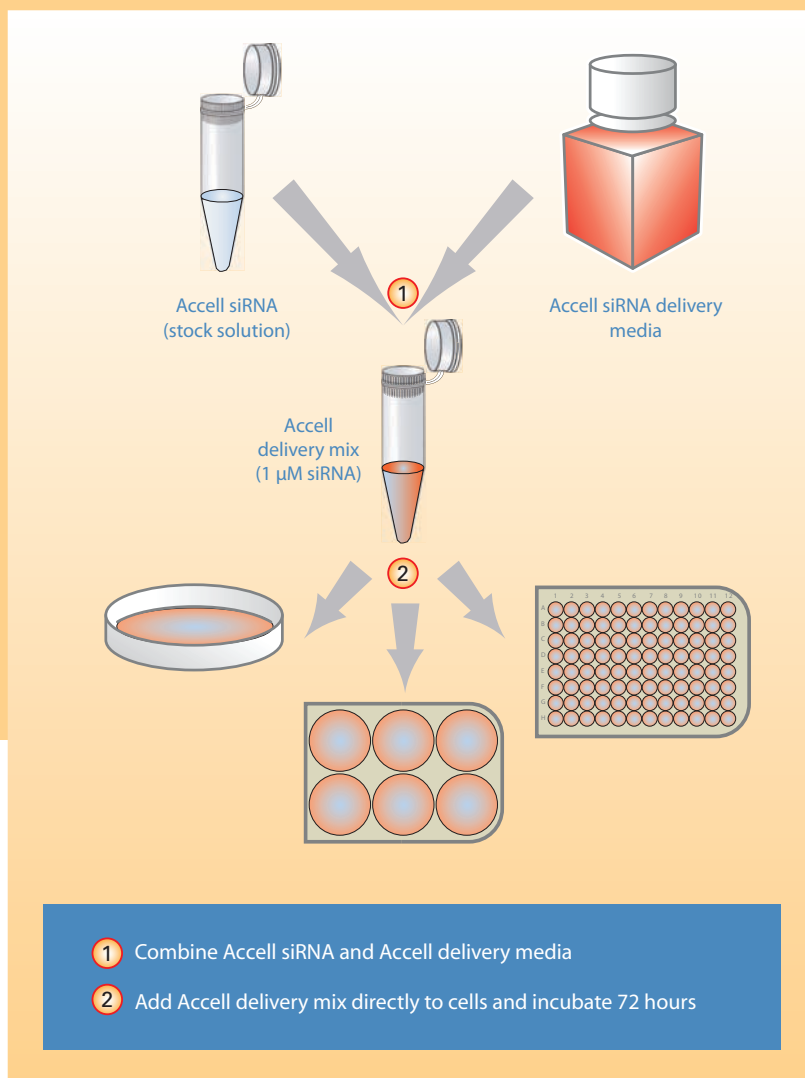
- Achieve faster time-to-results with a two-step delivery protocol
- Enjoy long-term reproducibility with Accell delivery mix stability of up to nine months at 4° C
- Simplify RNAi delivery conditions with a single, validated delivery media formulation\*

# ease-of-use

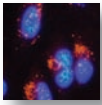
**Accell siRNA reagents deliver effective knockdown without cytotoxicity.** Eighteen cell lines were treated with 1  $\mu$ M Accell Cyclophilin B siRNA in Accell delivery media and assayed for knockdown at 72 hours.

mRNA expression was determined by QuantiGene branched DNA assay (Panomics) and cell viability was determined by alamarBlue (Biosource International).

### The Accell siRNA delivery protocol simplifies targeted gene knockdown



\* Accell siRNA is recommended for use with Accell delivery media (Cat# B-005000). This is a serum-free media specially formulated and optimized for use with Accell siRNA. It has undergone thorough testing and successful validation in over 40 cell lines. Other serum free/low serum media have also been used successfully with Accell siRNA. See protocol for details.



## Proven results in neuronal cells

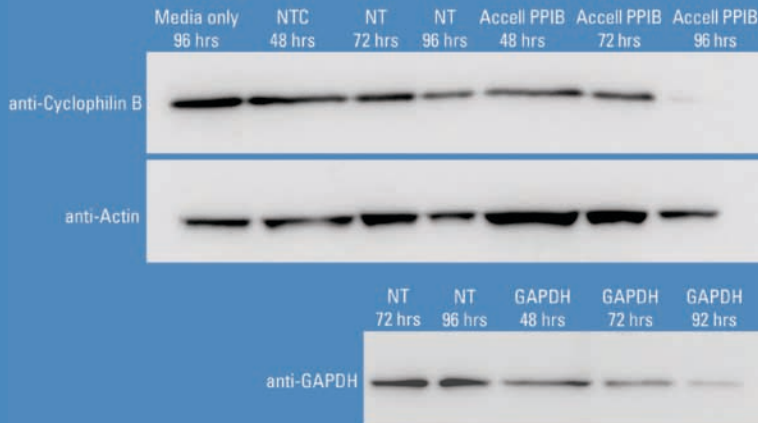
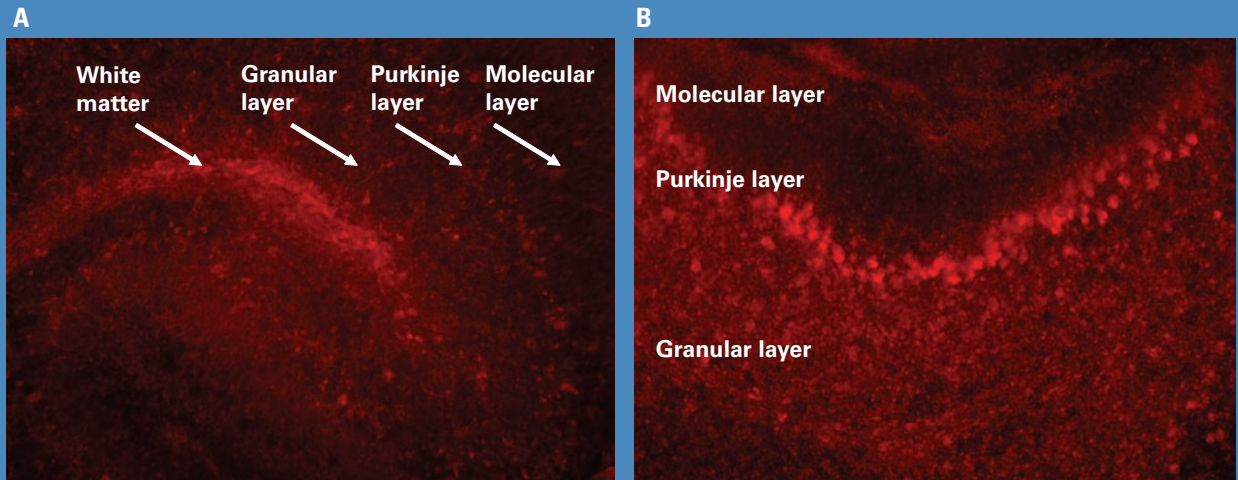
The ease and convenience of Thermo Scientific Accell siRNA application in cell types resistant to conventional transfection has enabled scientists, especially those in fields like immunology and neurobiology, to harness RNAi for a variety of applications.

- Medium- to high-throughput RNAi screens to assess gene function
- Application in organotypic cultures for monitoring of cellular behavior (Figure 1, 2)
- *In vivo* application for novel discoveries in live animal models\*

## novel discoveries

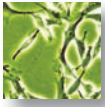
### Accell siRNA demonstrates effective uptake and silencing in organotypic brain slices

**Figure 1. Accell siRNA shows increased uptake with extended incubation.** 250 µm cerebellar sections were prepared, cultured and incubated for 3 hours (A) and 72 hours (B) with Accell Red Non-targeting control siRNA before inspection by microscopy.



**Figure 2. Accell siRNA effectively silences target genes in cultured P8 mouse brain slices.** Cultured brain slices were assessed following incubation with Accell siRNA targeting Cyclophilin B and GAPDH at three time points (48, 72, 96 hrs) and the level of remaining protein was evaluated using Western blot analysis (Actin as loading control).

\*DiFeo, A. et al. KLF6-SV1 is a novel antiapoptotic protein that targets the BH3-only protein NOXA for degradation and whose inhibition extends survival in an ovarian cancer model. *Cancer Res.* 69: 4733-41 (2009)  
 Gonzalez-Gonzalez, E. et al. Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD). *Mol Ther.* 18(9): 1667-74 (2010)  
 Li, Q. et al. Silencing MAP Kinase-activated Protein Kinase-2 arrests inflammatory bone loss. *J. Pharmacol. Exp. Ther.* 336: 633-642 (2011)



## Novel delivery causes minimal cellular perturbation for unmatched clarity of results

Imagine your results free from the toxicity and inflammatory response typically associated with conventional lipid transfection reagents.

The passive nature of Thermo Scientific Accell siRNA uptake avoids adverse cellular responses that could interfere with analysis of target gene knockdown. This new paradigm for siRNA delivery offers:

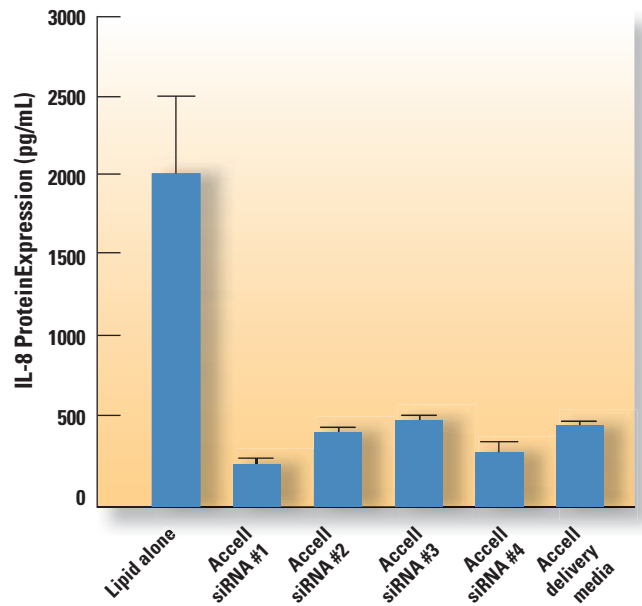
- No detectable inflammatory response
- Sustained viability at a range of cellular densities
- Elimination of non-specific lipid effects for pure silencing results

## clarity of results

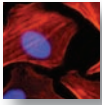
### Reduce off-targets and delivery effects with Accell siRNA



### Accell siRNA delivery does not induce expression of the IL-8 inflammatory protein



HeLa S3 cells were treated with either lipid alone (DharmaFECT 1 transfection reagent), 1  $\mu$ M Accell siRNA (either #1, 2, 3 or 4) targeting diazepam binding inhibitor or Accell delivery media alone. 72 hours after transfection, the cellular supernatant was analyzed for the cytokine IL-8 and eight other interferon response genes (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, IL-10, IL-12p70, IFN $\gamma$ , TNF $\alpha$ ) using the SearchLight array platform.



## Thermo Scientific Accell siRNA delivers a proven silencing strategy

Accell siRNA provides specific knockdown of your target gene by combining Thermo Scientific SMARTpool technology - recognized to enhance overall functionality and specificity - with additional proven strategies:

- Validated Thermo Scientific SMARTselection siRNA design algorithm for effective silencing
- Seed region filters for toxic and miRNA-like motifs along with seed frequency analysis to eliminate designs with high likelihood of off-target silencing
- Specificity-enhancing modifications on both strands to reduce off-target effects

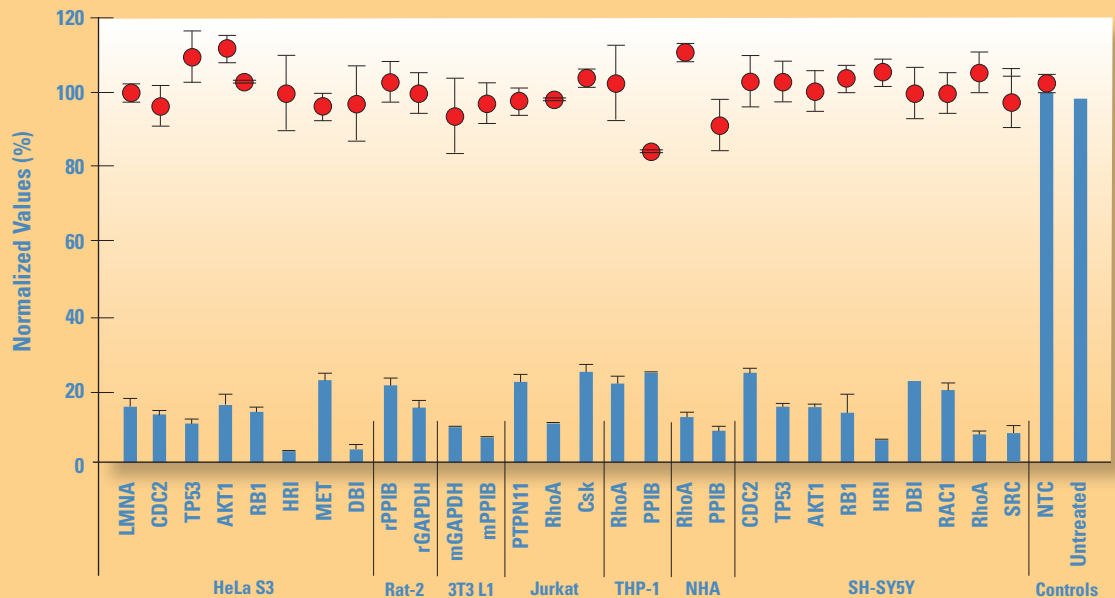
## proven silencing strategy

### Accell siRNA reagents successfully silence target genes across cell types

■ Target mRNA Levels  
● Cell Viability

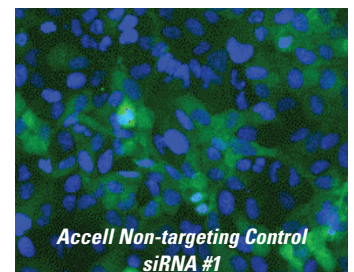
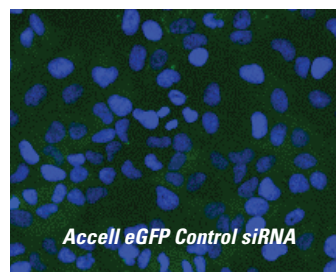
**Achieve effective target knockdown across cell types.** HeLa S3, Rat-2, 3T3L1, Jurkat, THP-1, NHA, and SH-SY5Y cells were treated with 1  $\mu$ M Accell SMARTpool siRNA targeting various genes or Accell Non-targeting control (NTC) and assayed for knockdown at 72 hours.

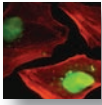
mRNA expression was determined by QuantiGene branched DNA assay (Panomics) and cell viability was determined by alamarBlue (Biosource International).



### eGFP expression silenced by Dharmacon Accell siRNA

FKHR-U2OS cells (BioImage) were treated with 1  $\mu$ M Accell eGFP Control siRNA or Accell Non-targeting Control siRNA #1 in Accell delivery media. Reduction of eGFP expression is visualized using Cellomics ArrayScan V<sup>1</sup> (green= GFP expression; blue= nuclear Hoechst dye).





## New delivery technology. New possibilities.

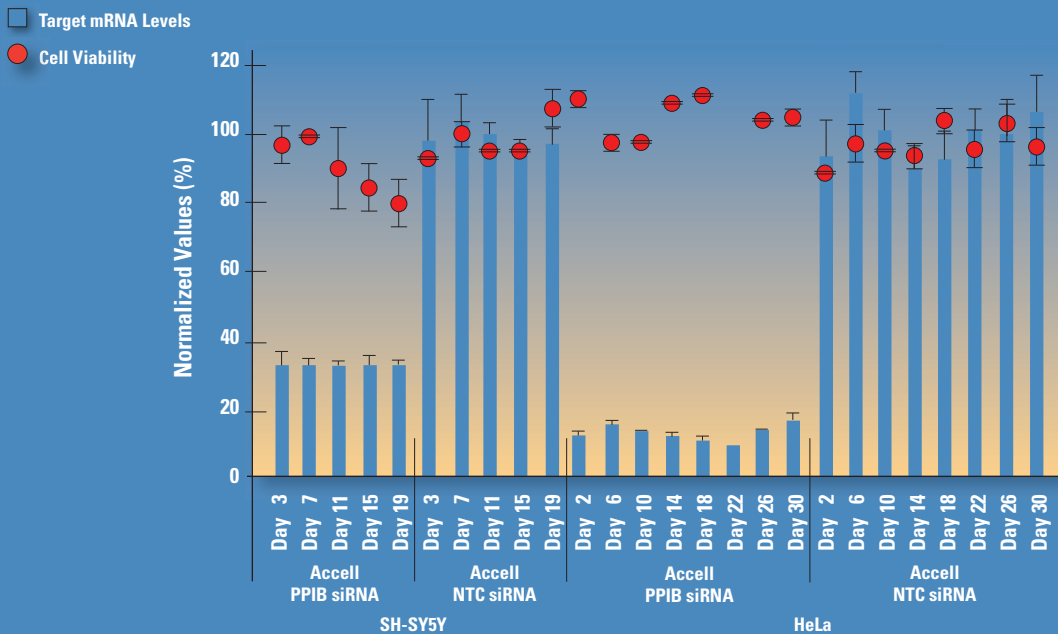
A new world of RNAi is waiting.

The passive nature of Thermo Scientific Accell siRNA uptake lends itself to gene silencing applications not possible with other delivery techniques:

- Repeated dosing for extended duration knockdown - useful for study of long-lived proteins and downstream cellular responses
- Transient silencing of multiple genes with multiplex delivery
- Combinatorial approaches for gene modulation using vector-based RNAi or over-expression technologies

# new possibilities

*Demonstration of extended duration silencing with continuous dosing of Accell siRNA*



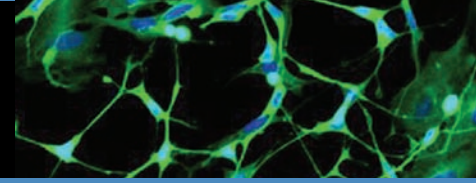
*HeLa and SH-SY5Y cells were treated with Accell Cyclophilin B Control siRNA at multiple intervals for sustained target knockdown. HeLa cells showed sustained knockdown for 30 days (9 passages), SH-SY5Y for 20 days (5 passages). At each passage, cells were split and cultured in the Accell delivery mix. Target knockdown was reassessed at 24 (HeLa) or 48 hr (SH-SY5Y) intervals following each Accell siRNA treatment.*

*mRNA expression was determined by QuantiGene bDNA assay (Panomics) and cell viability was determined by alamarBlue (Biosource International, Inc).*

Get started at [www.thermoscientific.com/dharmacon](http://www.thermoscientific.com/dharmacon)

- Predesigned Dharmacon Accell siRNA reagents** – Simply search for your human, mouse, or rat gene and click to order
- Dharmacon Accell siRNA delivery media** – Optimized for use with Dharmacon Accell siRNA to virtually eliminate delivery optimization
- Dharmacon Accell RNAi controls** – Validated positive controls for Cyclophilin B, GAPD, and eGFP, and non-targeting negative controls for high confidence results
- Dharmacon Accell siRNA libraries** – Check our website for a growing list of available gene collections

# Thermo Scientific Dharmacon Accell siRNA



**For further information visit:**

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Euro Literature # PB 2007 75

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