Knockdown and detection of long noncoding RNA

- Comprehensive pre-designed reagents for investigation of human IncRNAs
- siRNAs with enhanced specificity from the RNAi leaders
- High-efficiency qPCR assays for specific and dependable IncRNA detection
Detection and silencing of long noncoding RNA

Thermo Scientific™ Lincode™ siRNA reagents leverage the chemistry and RNAi expertise that have made Thermo Scientific Dharmacon siRNAs the world’s most trusted. Paired with Thermo Scientific™ Solaris™ qPCR IncRNA Expression Assays, you can achieve high-confidence knockdown and detection of long noncoding RNA.

Long noncoding RNA (lncRNA) has recently gained attention as a new class of regulatory RNA; with key roles in epigenetics, transcriptional regulation, development, cancer, neurological disorders, and other essential biological processes. Effective tools to silence lncRNAs will more fully elucidate the role of these molecules in genetic pathways.


Which human IncRNAs do our reagents target?

Lincode siRNAs and Solaris IncRNA qPCR assays target human noncoding RNA genes in the RefSeq v.54 database that meet the following criteria:

- ≥ 200 nt in length
- Gene record contains at least one RefSeq Accession prefix of NR_ or XR_
- Designation as IncRNA or miscellaneous RNA. Genes characterized as tRNA, rRNA, snoRNA, etc. are excluded from these product lines

What about long noncoding variants of protein coding genes?

Approximately 1000 human genes contain at least one non-coding (NR) transcript in addition to a protein-coding transcript (NM or XM). Lincode siRNAs have been developed to specifically target these non-coding transcripts when unique sequence space is available.
Lincode siRNA Reagents

Thermo Scientific Lincode siRNAs leverage the design tools, chemistry, and technology that have made Thermo Scientific™ siGENOME™ and ON-TARGETplus™ siRNA reagents the most-cited siRNAs in the world.

- Designed with the Thermo Scientific™ SMARTselection™ algorithm to ensure high-efficiency silencing
- Comprehensive BLAST analysis to ensure no sequence complementarity to any protein-coding (NM, XM) or other non-coding (NR, XR) transcript in RefSeq v54
- Lincode siRNAs carry proprietary dual-strand chemical modifications (ON-TARGETplus™) to ensure optimal strand loading for the reduction of off-targets
- Thermo Scientific™ SMARTpool™ siRNA reagents target multiple regions of the lncRNA to improve the likelihood of RNAi-mediated degradation

Why are high-confidence siRNA reagents so important for lncRNA knockdown studies?

lncRNA may be more difficult to silence than protein-coding genes due to:

- Nuclear localization of the lncRNA, reducing availability to RNAi cellular machinery
- Tertiary structure of the lncRNA, preventing siRNA access to the target region
- DNA or protein binding to the lncRNA, preventing siRNA access to the target region

Detection of effective lncRNA knockdown following application of Lincode siRNA reagents. (A) Knockdown of CDKN2B-AS1 in HeLa cells. (B) Knockdown of BDNF-AS1 in hNDF cells. All siRNAs were used at 25 nM. lncRNA transcripts were detected with Solaris qPCR lncRNA Expression Assays and normalized to Non-targeting Control siRNA. Viability was assessed by resazurin assay.

Learn more and perform a search for products targeting your lncRNA of interest at thermoscientific.com/lncRNA
Thermo Scientific Lincode siRNA screening libraries

Unbiased RNAi screens are important tools for elucidation of novel biological pathways and disease progression. The assessment of potential roles for microRNA and IncRNA, in addition to protein-coding genes, can provide a more complete picture of the genes involved in a phenotype of interest. Lincode siRNA screening libraries are offered as two separate collections based on NCBI RefSeq v.54:

- NR IncRNA – 1860 IncRNA gene records containing at least one NR accession number, plus siRNAs targeting IncRNA transcripts within protein-coding genes (371 targets)
- XR IncRNA – 1193 IncRNAs whose gene record contains at least one XR accession number (transcript products provided by genome annotation)

Lincode siRNA reagents are also available as custom libraries for any customer-defined collection to target IncRNAs of interest. Custom libraries may also include reagents from Thermo Scientific siRNA and microRNA product lines (ON-TARGETplus, Accell™, siGENOME™, and miRIDIAN™ pre-designed RNAi reagents).

### Human Lincode siRNA Library

<table>
<thead>
<tr>
<th>Library Type</th>
<th>Cat #</th>
<th># IncRNAs Targeted</th>
<th>Quantity per Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR IncRNA RefSeq v54 - SMARTpool</td>
<td>G-301000-xx</td>
<td>2231</td>
<td>0.1, 0.25, or 0.5 nmol per well</td>
</tr>
<tr>
<td>NR IncRNA RefSeq v54 - Set of 4</td>
<td>GU-301000-xx</td>
<td></td>
<td>96- or 384-well plate</td>
</tr>
<tr>
<td>XR IncRNA RefSeq v54 - SMARTpool</td>
<td>G-301100-xx</td>
<td>1193</td>
<td></td>
</tr>
<tr>
<td>XR IncRNA RefSeq v54 - Set of 4</td>
<td>GU-301100-xx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Custom Lincode siRNA Library</td>
<td>NA</td>
<td>Minimum of 20 wells</td>
<td>0.1, 0.25, 0.5, 1, or 2 nmol per well. Multiple plate replicates available</td>
</tr>
</tbody>
</table>

**SiRNA Screening Workflow**

1. **Select a Lincode siRNA Library targeting IncRNAs of interest**
   - All NR genes
   - All XR genes
   - Custom siRNA library targeting IncRNA of your choosing
2. **Transfect cells with siRNA reagents in Library**
   - DharmaFECT Transfection Reagent
   - Lincode Non-targeting Controls
3. **Perform biological assay to determine role of IncRNA in phenotype of interest**
4. **Verify depletion of IncRNA expression in potential hits**
   - Solaris qPCR IncRNA Expression Assay
   - Solaris Master Mix
   - GeneJET RNA Purification kit
   - Maxima cDNA synthesis kit
Many lncRNAs are transcribed from the opposite strand of protein-coding genes (Figure 3B). Therefore, it is of critical importance to ensure that siRNAs targeting these antisense lncRNA transcripts do not also silence a complementary protein-coding transcript.

The passenger strand-blocking features of the Lincode siRNA modification pattern (Figure 2) demonstrates effective silencing of the desired lncRNA while having little measurable effect on the mRNA from the opposite strand (Figure 3A).

**Figure 2.** Lincode siRNAs are modified with the proprietary ON-TARGETplus dual-strand modification that improves siRNA functionality by preferential loading of the guide strand into RISC. Additionally, off-targets are reduced due to:
- Inactivation of passenger strand activity
- Novel seed region modifications for disruption of microRNA-like off-targets

**Figure 3.** Sense strand activity is prevented by siRNA modifications.

(A) Lincode siRNA effectively knocks down BDNF-AS1 IncRNA, but not the protein-coding transcript BDNF that is antisense to the IncRNA target. This indicates strand specificity of Lincode siRNAs and effectiveness of ON TARGETplus modifications. siRNAs were applied at the indicated concentrations to hNDF cells. BDNF-AS1 and BDNF transcripts were detected with Solaris qPCR Expression Assays and normalized to Non-targeting control siRNA. Viability was assessed by resazurin assay and normalized to Untreated.

(B) Genomic context and Lincode siRNA targeting of BDNF-AS1 IncRNA. BDNF-AS1 IncRNA is antisense to BDNF protein-coding RNA. Direction of transcription from genomic DNA is indicated by arrows, exons are indicated by rectangles. Position of the Lincode siRNA target is indicated by double green lines.
Innovative designs to detect all IncRNA transcript variants with a single assay

Thermo Scientific Solaris qPCR Expression Assays are pre-designed to specifically detect long noncoding RNA transcripts in your cells of interest.

These high-performance assays are ideal for confirmation of gene silencing using Lincode siRNAs, and for smaller expression profiling studies. qPCR-based expression profiling allows for rapid, low-cost evaluation of a distinct set of IncRNAs.

Figure 4. Schematic representation of a Solaris probe fluorescing when bound to a specific target. (A) When a Solaris probe is in a free, unbound state, the FAM reporter and Eclipse™ Dark Quencher are in close proximity to one another and fluorescence is quenched. (B) When a Solaris probe binds to a denatured target the reporter and quencher are separated and fluorescence can be detected. Advancing DNA polymerase subsequently displaces the probe and restores quenching to reduce background signals.

Figure 5. Expression analysis of nine lncRNAs varies widely across three human cell lines. Fifty nanograms of total RNA was converted into cDNA using the Maxima First Strand cDNA Synthesis Kit (Cat #K1641). Standard RT-qPCR cycling conditions resulted in initial Cq values ranging from 22 (PPIB- protein-coding, reference gene) to 36 (BDNF-AS1, long noncoding RNA). Cell lines tested were HeLa, HEK293T and Human Neonatal Dermal Fibroblast (hNDF). lncRNA expression levels were variable among each cell line. lncRNA MLK-7-AS1 expression was not detectable in any of the cell lines tested.

Minor Groove Binder (MGB)

acts as a molecular anchor to strengthen the association between the probe and template, allowing shorter probes to be used for greater design flexibility.

Superbases

are incorporated to improve hybridization efficiency and prevent the formation of quadruplex structures as a result of self-annealing template, allowing shorter probes to be used for greater design flexibility.
Thermo Scientific Solaris IncRNA expression assays

Solaris qPCR IncRNA expression assays demonstrate highly efficient and specific detection of IncRNAs.

Figure 6. Validation of Solaris qPCR IncRNA Expression Assays. (A) Solaris assays efficiently amplify synthetic templates. A DNA template designed specifically for the CDKN2B-AS1 Solaris assay was amplified using standard Solaris qPCR protocols. A 10-fold dilution series was performed from $25 \times 10^6$ copies of synthetic template. Assay efficiency and dynamic range were determined using the resulting Cq values. (B) Solaris assays efficiently amplify endogenous templates. The CDKN2B-AS1 Solaris assay was tested using cDNA from the indicated cell types. A 10-fold dilution series was performed from 100 ng of total RNA input. Dynamic range and copy number varied by cell type.

<table>
<thead>
<tr>
<th>Solaris Assay</th>
<th>Cell Type</th>
<th>$R^2$</th>
<th>Efficiency</th>
<th>Dynamic Range</th>
<th>Copies / 100 ng RNA input</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2B-AS1</td>
<td>HEK293T</td>
<td>0.996</td>
<td>96.8 %</td>
<td>4/5</td>
<td>79317</td>
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<tr>
<td></td>
<td>HeLa</td>
<td>0.999</td>
<td>91.4 %</td>
<td>4/5</td>
<td>93450</td>
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<tr>
<td></td>
<td>hNDF(N)</td>
<td>0.995</td>
<td>94.3 %</td>
<td>3/5</td>
<td>71567</td>
</tr>
</tbody>
</table>

Figure 7. Solaris assays specifically amplify target genes. 5.0 ng of total RNA equivalents (lanes 2, 4, 6) or 0.5 ng of total RNA equivalents (lanes 3, 5, 7), from the indicated cell lines, were converted to cDNA and amplified with the CDKN2B-AS1 Solaris assay. Bands indicate single PCR products from endogenous templates. Ladder: Thermo Scientific™ FastRuler™ Ultra Low Range DNA Ladder (Cat #SM1233).

How many IncRNAs are there?

There are thousands of “non-coding RNA” entries in various databases, with many of these records undergoing a high rate of change. In order to create highly specific siRNA and qPCR reagents, Lincode and Solaris assays were pre-designed only for those genes with a high-confidence sequence record.
What about knockdown of a IncRNA that isn’t in RefSeq?

To design and order siRNAs targeting IncRNA that fall outside of the Lincode pre-designed products, follow these steps:
1. Go to the siDESIGN Center (thermoscientific.com/siDESIGN) and enter the RefSeq ID or nucleotide sequence of the IncRNA you wish to target
2. Select the BLAST database that includes NM and NR (coding and non-coding transcripts) for your species of interest
3. Add desired designs to your cart, then click each item to select ON-TARGET plus modifications to ensure strand-specific silencing

Still have questions? Need more assistance? Check out the online siDESIGN Guide or contact Technical Support.

Thermo Scientific Lincode siRNA Controls

As with microRNA modulation experiments, phenotypic responses due to silencing of IncRNA can be very subtle. Experimental RNAi controls are necessary to verify successful transfection and to control for non-specific effects. Lincode Non-targeting siRNAs are designed with three or more mismatches to every IncRNA and protein-coding gene in human, mouse, and rat. GASS is a widely expressed IncRNA whose knockdown has been demonstrated in multiple cell lines. It is recommended to determine GASS expression levels in your cell type prior to using the Lincode positive control. Alternatively, the use of ON-TARGET plus positive controls is an appropriate method for experimental optimization.

Lincode Negative Controls

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Available quantities</th>
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<tbody>
<tr>
<td>Lincode Non-targeting siRNA #1</td>
<td>D-001320-01-05, -20, -50</td>
<td></td>
</tr>
<tr>
<td>Lincode Non-targeting siRNA #2</td>
<td>D-001320-02-05, -20, -50</td>
<td></td>
</tr>
<tr>
<td>Lincode Non-targeting siRNA #3</td>
<td>D-001320-03-05, -20, -50</td>
<td></td>
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<tr>
<td>Lincode Non-targeting siRNA #4</td>
<td>D-001320-04-05, -20, -50</td>
<td></td>
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<tr>
<td>Lincode Non-targeting Pool</td>
<td>D-001320-10-05, -20, -50</td>
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Lincode Positive Controls

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Available quantities</th>
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<tbody>
<tr>
<td>Lincode GASS Control siRNA</td>
<td>D-001310-01-05, -20, -50</td>
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<tr>
<td>Lincode GASS Control Pool</td>
<td>D-001310-10-05, -20, -50</td>
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Note: Lincode Non-targeting controls are identical to ON-TARGET plus Non-targeting controls.

Product availability

All pre-designed Lincode siRNAs and Solaris assays are available via the online GENEius Search found at thermoscientific.com/GeneSearch. Simply search for your gene with standard identifiers and click to view and order the available products.

Lincode pre-designed Human siRNA reagents: Available Product Formats

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Available quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMARTpool</td>
<td>A mixture of 4 siRNA provided as a single reagent; providing advantages in both potency and specificity.</td>
<td>R-HUMAN-XX</td>
</tr>
<tr>
<td>Set of 4 Upgrade</td>
<td>Discounted price for the purchase of the four siRNAs targeting the same IncRNA.</td>
<td>RU-HUMAN-XX</td>
</tr>
<tr>
<td>Individual siRNAs</td>
<td>Select 1, 2, or 3 individual siRNAs per gene. Minimum purchase of four items at the 2 nmol size across all siRNA product lines.</td>
<td>N-HUMAN-XX</td>
</tr>
</tbody>
</table>

Solaris qPCR IncRNA Expression Assays: Available Product Formats

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat #</th>
<th>Available quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual IncRNA detection assays</td>
<td>All components provided at a 20X concentration (two primers at 800 nM ea and one gene-specific probe at 200 nM).</td>
<td>AZ-INVHSA-XX, AZ-BTOHSA-XX</td>
</tr>
</tbody>
</table>

These are agnostic product identifiers. Actual catalog numbers are gene-specific (e.g. R-003284-00) xx = quantity-specific catalog number suffix.

thermoscientific.com/IncRNA

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