October 2017

# Antisense LNA® GapmeRs Handbook

## LNA-optimized oligonucleotides for strand-specific knockdown of mRNA and lncRNA



QIAGEN

# Contents

# Kit Contents

| Product | Cat. no. | Label | Amount supplied |
|---|---|---|---|
| Antisense LNA GapmeR Standard | 339510<br>339511<br>339512 | No label or<br>5′ or 3′ FAM<br>for 5 and 15<br>nmol scales | 1, 5 or 15 nmol<br>oligonucleotide, dried down<br>in tube format |
| Antisense LNA GapmeR Premium | 339517<br>339518 | No label or<br>5′ or 3′ FAM | 5 or 15 nmol<br>oligonucleotide, dried down<br>in tube format |
| Antisense LNA GapmeR *in vivo* Ready | 339523<br>339524 | No label or<br>5′ or 3′ FAM | 5 or 15 nmol<br>oligonucleotide, dried down<br>in tube format |
| Antisense LNA GapmeR *in vivo* Large Scale | 339532 | No label or<br>5′ or 3′ FAM | Varies from 5 mg – 1 kg |
| Antisense LNA GapmeR Controls (5 nmol) | 339515<br>339516 | No label or<br>5′ or 3′ FAM | 5 or 15 nmol<br>oligonucleotide, dried down<br>in tube format |
| Antisense LNA GapmeR, custom plate | 339529<br>339530<br>339531 | No label or<br>5′ or 3′ FAM<br>for 5 and 15<br>nmol scales | 1, 5 or 15 nmol<br>oligonucleotide, dried down<br>in 96-well plate |

# Storage

Antisense LNA GapmeRs are shipped dried-down at room temperature. Unopened vials should be stored at –15 to –30ºC or below. Fluorescence-labeled oligonucleotides should be protected from light to avoid bleaching. When stored in this manner, they will remain stable at least 6 months after the shipping date. Exposure to higher ambient temperatures during shipment does not pose any risk to the stability of the oligonucleotides.

Oligonucleotides are degraded by repeated freeze-thaw cycles, especially when in solution. It is recommended to aliquot and store the GapmeRs at –15 to –30ºC or below in a constant-temperature freezer after re-suspension to avoid repeated freeze-thaw cycles. Do not store in frost-free freezers with automatic thaw-freeze cycles.

# Intended Use

Antisense LNA GapmeRs are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the product. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Antisense LNA GapmeRs is tested against predetermined specifications to ensure consistent product quality.

# Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

# Introduction

Antisense LNA GapmeRs are powerful tools for loss-of-function studies of proteins, mRNA and lncRNA (long noncoding RNA). These single-stranded, antisense oligonucleotides catalyze RNase H-dependent degradation of complementary RNA targets. Antisense LNA GapmeRs are 16 nucleotides long and are enriched with LNA in the flanking regions and DNA in a LNA-free central gap – hence the name "GapmeR" (Figure 1). The LNA-containing flanking regions increase target binding affinity, while also conferring nuclease resistance to the antisense oligo. When the GapmeR is hybridized to its target RNA, the central DNA gap activates RNase H cleavage of the opposing RNA strand. LNA GapmeRs have fully modified phosphorothioate (PS) backbones, which ensure exceptional resistance to enzymatic degradation.

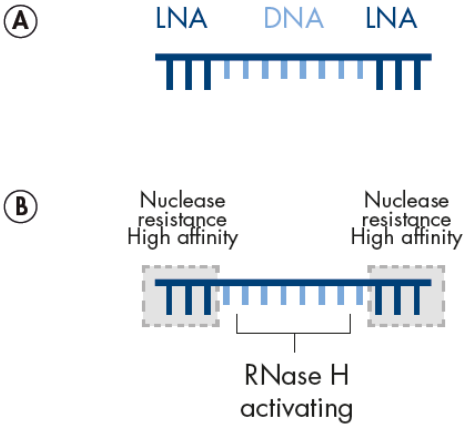


**Figure 1. A unique short, single-stranded antisense design.** (A) Antisense LNA GapmeRs are single-stranded, short 16mer oligonucleotides containing a DNA portion flanked by LNA. (B) The LNA parts increase the affinity for the target and confer nuclease resistance,

## Principle and workflow

Antisense LNA GapmeRs are potent antisense oligonucleotides primarily used to study the functions of mRNA or lncRNA by assessing the biological consequences of inhibiting their expression. Antisense LNA GapmeRs are designed with an empirically derived design algorithm. From our experience, we know that on average, approximately 2 out of 5 GapmeRs are capable of potent silencing of the intended target. Therefore, effective GapmeRs must be identified in an initial screening step. This typically involves measuring target RNA silencing by qPCR or similar methods. Once potent GapmeRs have been identified, they can be used to study the effect of silencing the target RNA in numerous ways, such as using cellular assays to monitor cell proliferation, cell differentiation or apoptosis. Observing the same phenotype with at least two different GapmeRs is a good control that the effects are due to antisense silencing of the intended target RNA and not due to unintended off-target effects.

## LNA technology

Locked nucleic acids (LNA) are a class of high-affinity RNA analogs in which the ribose ring is "locked" in the ideal conformation for Watson-Crick binding. As a result, LNA oligonucleotides exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand. For each incorporated LNA monomer, the melting temperature ($T_m$) of the duplex increases by 2–8ºC. In addition, LNA oligonucleotides can be made shorter than traditional DNA or RNA oligonucleotides and still retain a high $T_m$. This is important when the oligonucleotide is used to detect small or highly similar targets.

Since LNA oligonucleotides typically consist of a mixture of LNA and DNA or RNA, it is possible to optimize the sensitivity and specificity by varying the LNA content of the oligonucleotide. Incorporation of LNA into oligonucleotides has been shown to improve sensitivity and specificity for many hybridization-based technologies including PCR, microarray and *in situ* hybridization.

An LNA oligonucleotide offers substantially increased affinity for its complementary strand, compared to traditional DNA or RNA oligonucleotides. This results in unprecedented sensitivity and specificity and makes LNA oligonucleotides ideal for the detection of small or highly similar DNA or RNA targets.

# Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

Additional required materials:

- Nuclease-free TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5 or 8.0)
- Microcentrifuge
- DNase- and RNase-free microcentrifuge tubes or microtiter plate
- DNase- and RNase-free sterile filtered pipette tips
- Cell culture plates
- Cell culture medium
- Transfection reagent

# Protocol: Resuspension and Transfection

## Important notes before starting

Oligonucleotides are susceptible to degradation by exogenous nucleases introduced during handling. Wear powder-free gloves when handling the Antisense LNA GapmeRs. Use DNase- and RNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

## Resuspension of Antisense LNA GapmeRs

1. Briefly centrifuge the screw cap vial or 96-well plate at low speed (maximum 4000 *x g*) to ensure that all material is collected at the bottom of the vial or well before opening.
2. Carefully remove the screw cap or plate seal.
3. Add nuclease-free TE buffer to achieve the desired concentration. For a 50 µM solution, add 100 µl TE-buffer (10 mM Tris, pH 7.5 or 8.0, 0.1 mM EDTA) to 5 nmol of GapmeR.

   **Note**: Stock solutions of Antisense LNA GapmeRs should not be less than 10 µM.
4. Let the vial or plate stand for a few minutes at ambient temperature.
5. Gently pipette up and down 5 times to resuspend.
6. Repeat steps 4 and 5.
7. Aliquot the resuspended Antisense LNA GapmeRs into multiple tubes or plates to limit the number of freeze-thaw cycles. Store at –15 to –30°C or below. Working solutions can be stored at 2–8ºC for a maximum of 14 days.

   **Note**: Avoid freeze-thawing more than 5 times.

## Transfection guidelines

It is crucial to optimize the transfection conditions before screening for potent Antisense LNA GapmeRs and conducting experiments to observe effects of target RNA silencing.

Transfection efficiency varies according to the cell type and transfection reagent used. The optimal combination of cell type, transfection reagent and transfection conditions must be determined empirically. Optimizing transfection efficiencies is crucial for achieving potent target knockdown, while minimizing secondary effects. Expect to spend some time finding the optimal transfection conditions.

Optimal transfection conditions are found by identifying efficient transfection reagents for each cell line and by adjusting the following parameters:

- Amount of transfection reagent
- Amount of Antisense LNA GapmeR
- Cell density at the time of transfection
- Order of transfection (e.g., plating cells before transfection or plating cells at the moment of transfection)
- Length of exposure of the cells to transfection reagent/oligonucleotide complex

We strongly recommend using a validated Antisense LNA GapmeR positive control for optimization of transfection conditions. Several positive controls have been developed for different types of RNA targets that are expressed in a broad range of cell types.

Most protocols recommend maintaining mammalian cells in the medium used for transfection for 24 hours. The transfection medium should then be replaced with fresh medium to maximize viability of the cell culture. Normally, Antisense LNA GapmeRs display potent activity at final concentrations of 1–50 nM. The optimal time for analyzing the effect of transfection must be determined experimentally, but typically robust target knockdown is

observed after 24 hours. When performing phenotypical characterization always, use the lowest GapmeR concentration required to silence the target RNA, to minimize influence from any potential off-target activity.

**Table 1. General transfection guidelines.**

| Cell culture plate | 96 well | 24 well | 12 well | 6 well |
|---|---|---|---|---|
| Transfection reagent* | 0.3–1.0 µl | 1–3 µl | 2–4 µl | 3–36 µl |
| Antisense LNA GapmeR† | 5 pmol | 25 pmol | 50 pmol | 150 pmol |
| Cell density (cells/well)‡ | 6,000 | 40,000 | 80,000 | 240,000 |
| **Final volume per well** | 100 µl | 500 µl | 1000 µl | 3000 µl |

* Refer to the instructions provided by the transfection reagent supplier.

† The amount shown yields an Antisense LNA GapmeR concentration of 50 nM.

‡ Optimal cell density varies with the cell type depending on cell size and growth characteristics. In general, 30–70% confluency is recommended.

## Electroporation

Antisense LNA GapmeRs can also be introduced into cells by electroporation. This is especially useful with cells that are notoriously difficult to transfect (e.g., non-adherent cells, such a lymphocytes, bone marrow stem cells and primary cancer cells). Follow the instructions provided with your electroporation system.

## Unassisted uptake (gymnosis)

Antisense LNA GapmeRs have a fully phosphorothioate (PS)-modified backbone, which makes them highly resistant to enzymatic degradation. They are so stable and potent that they can be added directly to serum-containing culture medium without the need for transfection reagents. The short oligonucleotides are taken up naturally by cells, and efficient antisense activity can be achieved by such unassisted "naked" delivery, also known as "gymnosis" (1, 2). However higher concentrations are required, and the uptake kinetics are slower than when using transfection reagents. The efficiency of unassisted uptake is highly

dependent on the cell line. Normally, potent antisense activity is achieved at oligonucleotide concentrations between 100 nM – 5 µM, and the effects are typically observed only after 48–72 hours incubation. In general, unassisted delivery has no effect on cell viability or cell morphology and therefore allows you to study the consequences of miRNA silencing without having to worry about confounding side effects from the transfection reagents. Unassisted delivery is a useful alternative for difficult-to-transfect cells and when normal transfection procedures (addition of transfection reagents or electroporation) have unacceptable phenotypic consequences.

Prior to screening of GapmeRs, we recommend using validated positive control Antisense LNA GapmeRs for assessing the susceptibility of cell lines to unassisted uptake.

Optimal gymnotic conditions are determined by adjusting the following parameters:

- Amount of Antisense LNA GapmeR: the Antisense LNA GapmeR solution should be prepared at the final concentration (100 nM –5 µM) in the appropriate fresh culture media before plating the cells.
- Cell density: to avoid problems associated with over-confluency of cells, the plating cell density can be appropriately decreased when a long incubation period is anticipated.
- Length of exposure of cells to the Antisense LNA GapmeR: a general guideline for the incubation time is 1–6 days after initial treatment. If an incubation longer than 6 days is required, it is recommended to replace the culture media with fresh media containing the Antisense LNA GapmeR.

## References


1. Stein, C.A. et al. (2010) Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. Nucleic Acids Research **38(1)**:e3.
2. Soifer, H.S. et al. (2012) Silencing of gene expression by gymnotic delivery of antisense oligonucleotides. Methods Mol Biol. **815**:333.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Antisense LNA GapmeR Standard (1 nmol) | 1 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade | 339510* |
| Antisense LNA GapmeR Standard (5 nmol) | 5 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade | 339511* |
| Antisense LNA GapmeR Standard (15 nmol) | 15 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade | 339512* |
| Antisense LNA GapmeR Premium (5 nmol) | 5 nmol oligonucleotide, dried down in tube format; premium cell-culture grade | 339517* |
| Antisense LNA GapmeR Premium (15 nmol) | 15 nmol oligonucleotide, dried down in tube format; premium cell-culture grade | 339518* |
| Antisense LNA GapmeR *in vivo* Ready (5 nmol) | 5 nmol oligonucleotide, dried down in tube format; *in vivo* ready grade | 339523* |
| Antisense LNA GapmeR *in vivo* Ready (15 nmol) | 15 nmol oligonucleotide, dried down in tube format; *in vivo* ready grade | 339524* |
| Antisense LNA GapmeR *in vivo* Large Scale | Varies from 5 mg – 1 kg oligonucleotide; option of grades | 339532* |
| Antisense LNA GapmeR Controls (5 nmol) | 5 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade | 339515* |
| Antisense LNA GapmeR Controls (15 nmol) | 15 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade | 339516* |
| Antisense LNA GapmeR, custom plate (1 nmol) | 1 nmol oligonucleotide, dried down in 96-well plate; option of grades | 339529* |

* The exact product numbers vary, depending on the particular product ordered and its specifications.

| Product | Contents | Cat. no. |
|---|---|---|
| Antisense LNA GapmeR, custom plate (5 nmol) | 5 nmol oligonucleotide, dried down in 96-well plate; option of grades | 339530* |
| Antisense LNA GapmeR, custom plate (15 nmol) | 15 nmol oligonucleotide, dried down in 96-well plate; option of grades | 339531* |
| **Related products** | | |
| miRCURY LNA miRNA Inhibitor (5 nmol)† | 5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; normal phosphodiester bonds | 339121* |
| miRCURY LNA miRNA Power Inhibitor (5 nmol)† | 5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; phosphorothioate-modified backbone | 339131* |
| miRCURY LNA miRNA Family Power Inhibitor | 5 nmol oligonucleotide set, dried down in tube format; no label; phosphorothioate-modified backbone | 339160* |
| miRCURY LNA miRNA Power Target Site Blocker (5 nmol)† | 5 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone | 339194* |
| miRCURY LNA miRNA Power Target Site Blocker, *in vivo* ready (5 nmol)† | 5 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone | 339199* |
| miRCURY LNA miRNA PCR Assay | LNA-optimized PCR assay for miRNA quantification; for 200 reactions | 339306* |
| miRCURY LNA miRNA Custom PCR Assay | Custom-designed and LNA-optimized PCR assay for miRNA quantification; for 200 reactions | 339317* |

* The exact product numbers vary, depending on the particular product ordered and its specifications.

† Other product sizes available; visit **www.qiagen.com** for more details.

| Product | Contents | Cat. no. |
|---|---|---|
| miRCURY LNA RT Kit | 5x RT Reaction Buffer, 10x RT Enzyme Mix, UniSp6, RNA Spike-in template, RNase-free water; for 8–64 reactions | 339340 |
| miRCURY LNA miRNA PCR Starter Kit | 2 miRCURY LNA PCR Assays of your choice, UniSp6 Spike-in control assay, miR-103-3p endogenous control assay, 5x RT Reaction Buffer, 10x RT Enzyme Mix, UniSp6 RNA Spike-in template, RNase-free water, 2x miRCURY SYBR Green Master Mix; for 20 RT reactions and 100 PCR amplifications | 339320 |
| miRCURY LNA SYBR Green PCR Kit (200)† | 2x miRCURY SYBR Green PCR Master Mix, miRCURY SYBR Green PCR Buffer and dNTP mix (dATP, dCTP, dGTP, dTTP), ROX Reference Dye, Nuclease-free Water; for 200 reactions | 339345 |

* The exact product numbers vary, depending on the particular product ordered and its specifications.

† Other product sizes available; visit **www.qiagen.com** for more details.

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## Notes

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