

Locked Nucleic Acid[®] (LNA[®]) Fluorescent Probes for Real-Time Quantitative PCR

LNA[®] LightCycler[®] Probes
LNA Dual-Labeled Fluorogenic Probes
LNA Molecular Beacons

PROLIGO
PRIMERS & PROBES

Three Good Reasons to Order from Proligo Primers & Probes™

1. Quality

The efficiency of the synthesis procedure, in particular the base coupling efficiency, is the most significant factor to influence the final quality of an oligonucleotide, even if it is subsequently purified. Our patented automated synthesis platform provides superior coupling efficiency, enabling us to offer high quality oligonucleotides.

2. Purity

For no additional fee, all of our custom oligonucleotides are deprotected and desalted. This process is required to remove the by-products of synthesis, making our oligonucleotides suitable for use with most molecular biological applications. However, for more stringent applications, such as site-directed mutagenesis and cloning, additional purification of the oligonucleotide may be required to remove truncated failure (or N-) product. Purification can be achieved by RPC (Reverse Phase Cartridge), PAGE (Poly Acrylamide Gel Electrophoresis) or RP-HPLC (Reverse Phase-High Pressure Liquid Chromatography). Choice of purification grade depends largely on the application.

Note: Locked Nucleic Acid® (LNA®) fluorescent probes are deprotected, desalted and purified by PAGE or RP-HPLC.

3. Synthesis Yield

We guarantee the total final yield of an oligonucleotide as a minimum number of OD (optical density) units, rather than providing the scale of synthesis. The scale of synthesis is only the starting point for synthesis, as the post-synthesis yield can vary according to oligonucleotide length, sequence, purification, modifications and coupling efficiencies.

Design Service for LNA Fluorescent Probes

Save time by letting Proligo Primers & Probes™ design your LNA fluorescent probes and associated primers for real-time quantitative PCR¹ applications. Send your design query to oligo.design@proligo.com. See page seven for more information.

To Order

Please order on-line at www.proligo.com. Alternatively, complete an email or fax order form, available from our Web site or by contacting your local Proligo® representative.

When you order on-line or by email, you will receive an automatic order confirmation, by email, within a few hours. When your oligonucleotides are ready to be shipped, you will also receive notification by email.

We manage our customers' information with the highest degree of confidentiality.

Pricing

Please contact your local Proligo Primers & Probes' representative for pricing information.

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For more information about our DNA fluorescent probes or real-time quantitative PCR (Q.PCR) technology, please visit www.proligo.com or contact your local Proligo representative and ask for our brochure, Fluorescent Probes for Real-Time Quantitative PCR.

Corporate Profile of Proligo

The knowledge of genes and the understanding of their functions are the tools to open up a whole new dimension for the diagnosis and curing of diseases. Processing of genetic information is a molecular event that consumes nucleic acid chemicals. Proligo, a dedicated specialist in nucleic acid chemistry, produces the consumables needed to process genetic information. We continually strive to meet our customers' needs through the introduction of novel technologies.

Proligo has production facilities in North America, France, Germany, Japan, Singapore and Australia. Proligo operates two business lines that span the genomics development supply chain: from oligonucleotide synthesis reagents (Proligo Reagents™) to production of oligonucleotides (Proligo Primers & Probes™).

Locked Nucleic Acid (LNA) Fluorescent Probes for Real-Time Quantitative PCR

- *Increased thermal stability and hybridization specificity*
- *More accurate gene quantitation and allelic discrimination*
- *Easier and more flexible probe designs for problematic target sequences*

To overcome certain weaknesses of standard DNA probe chemistries, choose from our range of Locked Nucleic Acid® (LNA®) fluorescent probes, a new generation of sequence-specific powerful real-time quantitative PCR probes:

- LNA LightCycler probes™
- LNA dual-labeled fluorogenic probes™
- LNA molecular beacons™

LNA fluorescent probes are a novel alternative in real-time PCR or end-point analytical assays for the following applications:

- Gene expression profiling
- Mutation detection
- Allelic discrimination
- Single nucleotide polymorphisms (SNPs)
- Pathogen detection

We will design your LNA fluorescent probes for you. Read about our custom design service on page 7.

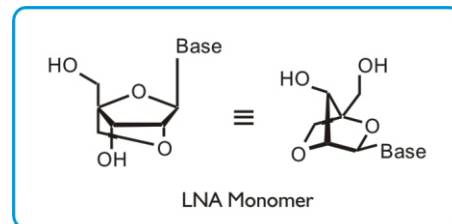
LNA fluorescent probes can be used for quantification, melting curves profiles, as well as in single, multiplexing or high-throughput screening assays.

All our LNA fluorescent probes are deprotected, desalted and purified by PAGE or RP-HPLC. They are also systematically quality-controlled by PAGE or MALDI-TOF mass spectrometry analysis.

What is LNA?

LNA is a novel type of nucleic acid analog that contains a 2'-O, 4'-C methylene bridge. This bridge—locked in 3'-endo conformation—restricts the flexibility of the ribofuranose ring and locks the structure into a rigid bicyclic formation, conferring enhanced hybridization performance and exceptional biological stability.

For more information about LNA, visit www.proligo.com or contact your Proligo representative.



The LNA Fluorescent Probe Advantage

Increased thermal stability and hybridization specificity

Introducing LNA chemistry into a real-time quantitative PCR (Q.PCR) probe increases thermal duplex stability² and improves specificity of probe hybridization to its target sequence³. As such, background fluorescence from spurious binding is reduced and the signal-to-noise ratio is increased.

The LNA monomer chemical structure enhances the stability of the hybridization of the probe to its target. As a result, the duplex melting temperature (T_m) may increase by up to 8°C per LNA monomer substitution in medium salt conditions—compared to a DNA fluorescent probe for the same target sequence—depending on the target nucleic acid⁴. This increase in hybridization creates a significant broadening in the scope of assay conditions and allows for more successful single-tube multiplexing⁵.

™ Patent pending

Further, it is possible to optimize the T_m level and the hybridization specificity through specific placement of the LNA base(s) in the probe design⁶.

Figure 1: An increase in the number of LNA bases within a Q.PCR probe increases T_m values

Probe Sequence (5' -> 3')	LNA base	T_m^*	ΔT_m	$\Delta T_m/\text{LNA}$
dG dT dG dA dT dA dT dG dC	-	29°C	-	-
dG +T dG dA +T dA +T dG dC	3	44°C	15°C	+5°C
+G +T +G +A +T +A +T +G +C	9	64°C	35°C	+3.9°C

* T_m of duplex between probe and its complementary DNA sequence

Note: + symbol denotes the LNA base.

More accurate gene quantitation and allelic discrimination

The ability of Q.PCR probes to readily discriminate between SNPs, the most abundant form of genetic variation, is greatly enhanced by the incorporation of LNA bases⁷⁻⁹.

Figure 2: LNA dual-labeled fluorogenic probes discriminate better than DNA dual-labeled fluorogenic probes in SNP genotyping analysis¹².

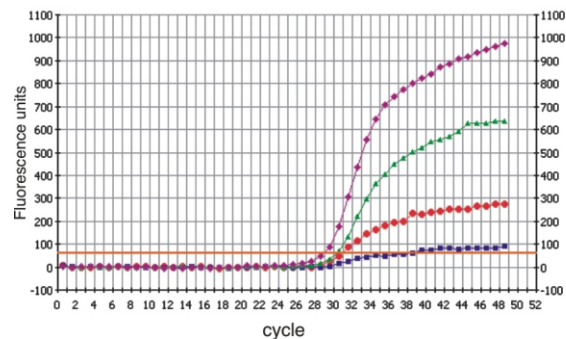
Key

Pink plots: Mutant-type DNA analysis with LNA mutant probe (16 mer with 3 LNA bases)

Green plots: Mutant-type DNA analysis with DNA mutant probe (25 mer)

Purple plots: Wild-type DNA analysis with LNA mutant probe (16 mer with 3 LNA bases)

Red plots: Wild-type DNA analysis with DNA mutant probe (25 mer)



Utilizing LNA for allelic discrimination is an extremely reliable and effective means for SNP-calling in genotyping applications. The presence of a single base mismatch has a greater destabilizing effect on the duplex formation between a LNA fluorescent probe and its target nucleic acid, than with a conventional DNA fluorescent probe. Shorter probes incorporating LNA bases can be used at the same temperatures as longer conventional DNA probes.

Easier and more flexible probe designs for problematic target sequences

Due to LNA's enhanced hybridization characteristics and then significant T_m contribution, LNA-containing Q.PCR probes can be synthesized to be shorter, allowing flexibility in design while still satisfying assay design guidelines. As such, certain design limitations that cannot be overcome with standard DNA probe chemistries can now be reduced or eliminated.

In addition, by using LNA fluorescent probes, shorter probes can be designed to address traditionally problematic target sequences, such as AT- or GC-rich regions. Also, the design of probes for querying difficult or inaccessible SNPs, such as the relatively stable G:T mismatch, is greatly facilitated by LNA.

For example, AT-rich Q.PCR probes often need to be over 30 bases long (and sometimes over 40 bases) to satisfy amplicon design guidelines—but may still perform poorly. With LNA fluorescent probes, the selective placement of LNA base substitutions facilitates the optimal design of highly specific, shorter probes that perform very well, even at lengths of 13 to 20 bases.

Compatible with real-time PCR platforms and end-point analytical detection instruments

LNA fluorescent probes are compatible with real-time PCR platforms and end-point analytical detection instruments, depending on the excitation/emission wavelengths of the dyes and the equipment. This gives you the freedom to work with the instrumentation and reagents platform of your choice under universal cycling conditions. No additional capital expenditure for specialized equipment is required.

LNA monomers are incorporated by the same standard phosphoramidite chemistry as DNA and RNA monomers

LNA-containing oligonucleotides are amenable to the same synthesis and modification protocols as DNA and RNA bases. They are soluble in standard buffers and water, and follow basic Watson-Crick base-pairing rules¹⁰.

Features of LNA Fluorescent Probes

- **Higher T_m by LNA contribution** increases thermal stability and hybridization specificity
- **More accurate gene quantitation and allelic discrimination** improves SNP detection assays
- **Shorter LNA probes** enable easier and more flexible probe designs for problematic target sequences
- **Compatible with real-time PCR platforms and end-point analytical detection instruments** - no additional capital investment is required
- **Incorporation of LNA monomers by standard phosphoramidite chemistry** allows the use of the same synthesis/modification protocols as DNA and RNA bases¹⁰

LNA LightCycler Probes

For your convenience, LNA LightCycler probes are available at a fixed price per oligonucleotide, within the following parameters:

- Up to 6 LNA bases
- Deprotected, desalted and purified by RP-HPLC
- Available in lengths of 7 to 40 mers
- Delivered dried in individual, opaque tubes
- Shipped in 6 working days of receiving your order, pending successful QC validation*

Guaranteed yields of LNA LightCycler probes		
Guaranteed yield (OD)	Approximate yield (nmols)	Estimated assays (reactions)
0.1	0.3	100
0.25	1	300
1.5	3	1,000

Please enquire for alternative quantities.

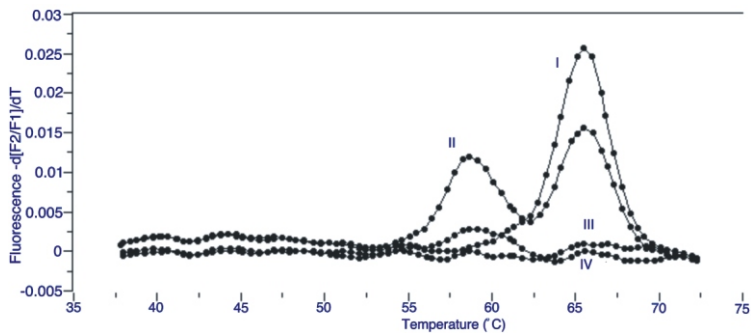
Labels and modifications for LNA LightCycler probes			
	LNA Probe 1	LNA Probe 2	
	3'-end donor fluorophore	3'-end	5'-end acceptor fluorophore
Labels and modifications	LightCycler Fluorescein	Phosphate	LightCycler Red 640

Note: Fluorescent signaling occurs during the annealing step of Q.PCR.

Figure 3: Melting curve profiles for Human Cystic Fibrosis SNP G542X demonstrate the superior discrimination power of LNA LightCycler probes over DNA LightCycler probes.

Figure 3a: A set of DNA LightCycler probes (A-25; D-22)

- Key**
- I Wild-type template
 - II Heterozygous template
 - III Mutant-type template
 - IV Control without template

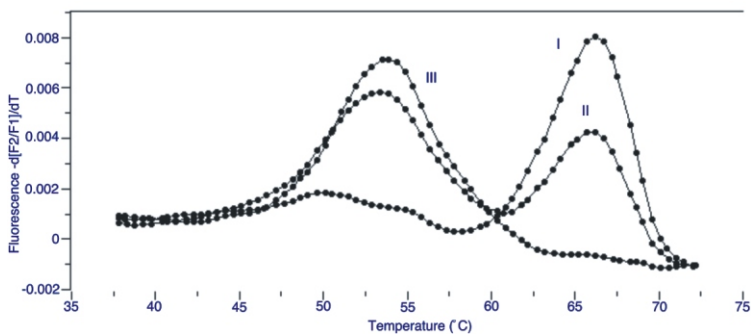


* Please check with your Prologo representative for local delivery times.

Figure 3b: A set of LNA LightCycler probes (A-16; D-16)

Key

- I Wild-type template
- II Heterozygous template
- III Mutant-type template



Note the DNA LightCycler probes do not pick up on the presence of the homozygous mutant, whereas the LNA LightCycler probes are able to identify the presence of all three template types, including the homozygous mutant.

LNA Dual-Labeled Fluorogenic Probes and LNA Molecular Beacons

For your convenience, LNA dual-labeled fluorogenic probes and LNA molecular Beacons are available at a fixed price per oligonucleotide, within the following parameters:

- Up to 8 LNA bases
- Deprotected, desalted and purified by PAGE or RP-HPLC
- Available in lengths of 7 to 40 mers
- Delivered dried in individual, opaque tubes
- Shipped in 7 working days of receiving your order, pending successful QC validation*

Guaranteed yields of LNA dual-labeled fluorogenic probes and LNA molecular Beacons		
Guaranteed yield (OD)	Approximate yield (nmols*)	Estimated assays (reactions [∅])
1	5	500
5	25	2,500
10	50	10,000

* Estimate 1 OD = 5 nmols = 30 µg, for a 20 mer oligo

∅ Estimated assay reactions for a 50 µl reactional volume.

Please enquire for alternative quantities.

Labels for LNA dual-labeled fluorogenic probes and LNA molecular Beacons	
5'-end reporter	3'-end quencher
6-FAM™, HEX™ or TET™	TAMRA™, DABCYL, BHQ™-1 or BHQ-2

Note: For LNA dual-labeled fluorogenic probes, fluorescent signaling occurs during the elongation step of Q.PCR. For LNA molecular Beacons, this occurs during the annealing step.

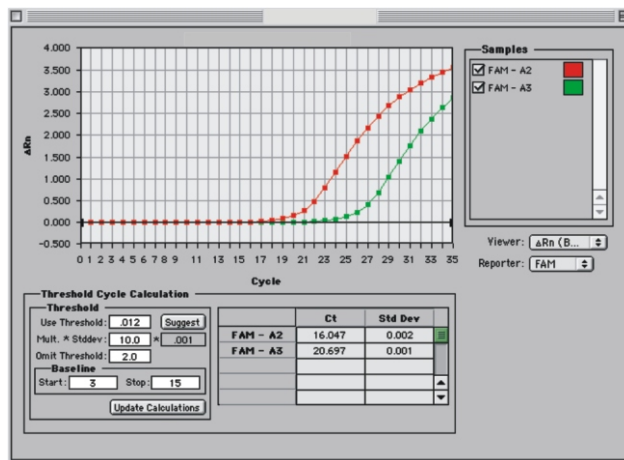
*Please check with your Proligo representative for local delivery times.



Figure 4: LNA dual-labeled fluorogenic probes vs. TaqMan® MGB probes in 5' nuclease PCR assays. (Amplification of *staphylococcal enterotoxin* gene in a *Staphylococcus aureus* strain¹¹)

Key

- LNA dual-labeled fluorogenic probe
- TaqMan MGB probe



Specifications of LNA Fluorescent Probes for Real-Time Quantitative PCR

Quality control

All our oligonucleotides undergo vigorous process monitoring and strict quality control. Length and labeling are systematically controlled by PAGE or MALDI-TOF mass spectrometry analysis. Quantity is systematically determined and validated by UV Absorbance at 260 nm.

Fluorescent probes are systematically quality-controlled by RP-HPLC. Additionally, the ratio between dye absorbance (nm) and DNA/RNA absorbance (nm) is calculated for the main product peak. This ratio defines the purity of the labeled probe, demonstrating that no contamination from partially labeled or unlabeled probes exists.

Purification

Fully deprotected and desalted. Purified by PAGE or RP-HPLC

Length

7 to 40 mers (excluding labels and/or modifiers)

Bases

DNA/LNA chimeras:

- LNA (+A, +C, +G or +T) (+ denotes the LNA base)
- DNA (A, C, G or T)

LNA LightCycler probes can contain up to 6 LNA bases

LNA dual-labeled fluorogenic probes and LNA molecular beacons can contain up to 8 LNA bases

Backbone

Phosphodiester bond

Format

Delivered in dry form, in opaque tubes

Turn-around time (TAT)

LNA LightCycler probes:	6 working days
LNA dual-labeled fluorogenic probes:	7 working days
LNA molecular beacons:	7 working days

Turn-around time is dependent upon successful QC validation and does not include shipping time. Please check with your Proligo representative for local delivery times.

Storage and stability

Although oligonucleotides can remain stable in solution at 4°C for up to two weeks, Proligo strongly recommends storage at -20°C. Repetitive freeze-thaw cycles should be avoided by storing as aliquots. Storing at concentrations above 20 μM is recommended under -20°C in TE Buffer. Oligonucleotides with fluorescent labels should be protected from light. Proligo guarantees its oligonucleotides for six months, when stored under the above conditions.

Shipment

Shipped by mail or express delivery, dry, in individual, opaque tubes

Oligonucleotide Technical Data Sheet

Oligonucleotides are delivered with an Oligonucleotide Technical Data Sheet, which includes oligonucleotide name, sequence, concentration, precise quantity in OD and nmols, T_m, MW, size, extinction coefficient and purification data.

RP-HPLC profile

Oligonucleotides are delivered with an RP-HPLC profile for each LNA fluorescent probe

Services available upon request

- Custom design service
- Aliquoting

Note: Additional services may increase turn-around time.

Pricing

Please contact your local Proligo representative.

Ordering

On-line at www.proligo.com, by email or by fax

Spectral Properties of Fluorophores

The table below details the dyes used for the LNA fluorescent probes, together with their excitation and emission data. Please enquire for any other labels.

Quenchers	Maximum absorbance (nm)	Maximum emission (nm)
BHQ-1 Dark Quencher	535	None
BHQ-2 Dark Quencher	579	None
DABCYL Dark Quencher	453	None
TAMRA	550	576

Labels	Maximum absorbance (nm)	Maximum emission (nm)
6-FAM	495	517
HEX	537	553
LightCycler Fluorescein	495	520
LightCycler Red 640	625	640
TET	521	538

Design Service for LNA Fluorescent Probes[†]

Save time by letting Proligo Primers & Probes design your LNA fluorescent probes and associated primers for your real-time quantitative PCR applications e.g. gene expression profiling, SNP genotyping, melt curve profiling, pathogen identification. We offer design for:

- LNA LightCycler probes
- LNA dual-labeled fluorogenic probes
- LNA molecular beacons

Through extensive research—by means of collaborations with experienced scientists in the field of Q.PCR, and through our own internal research—we have the knowledge and experience to recommend optimal LNA Q.PCR designs. Our LNA probe design software will analyze multiple interactions between the probe, primers and generated amplicon, and identify the best placement for your LNA base(s).

Should you work with SYBR[®] Green I and need primer sequences, we recommend that we also design the appropriate LNA fluorescent probe(s) at the same time.

Our team of design specialists is always available to answer any questions you may have. Please contact us at oligo.design@proligo.com.

References

1. PCR (Polymerase Chain Reaction) is patented by Hoffman La Roche Ltd.
2. Braasch, D., S. Jensen, Y. Liu, K. Kaur, K. Arar, M. White, and D. Corey. RNA interference in mammalian cells by chemically-modified RNA. *Biochemistry*. 8:42(26):7967-75, 2003.
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12. Ugozzoli, L., D. Latorra, R. Pucket, K. Arar, and K. Hamby. Genotyping in real-time by oligonucleotide probes containing locked nucleic acids (LNA). *Analytical Biochemistry*. In press.

OD = optical density

Specifications are subject to change. Please refer to our Web site at www.proligo.com for the latest details.

[†]Note: Functionality of the designs chosen by our design software cannot always be guaranteed.

All products are sold for research use only.

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Side Note

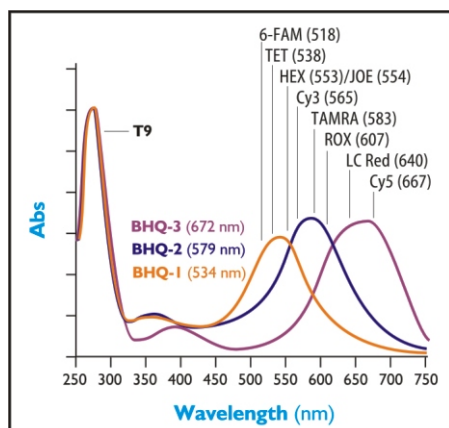
What are Black Hole Quenchers™?

Some commonly used quenchers—such as DABCYL and TAMRA—suffer from a number of drawbacks, including poor spectral overlap between the fluorescent dye and quencher molecule (DABCYL) or inherent fluorescence of the quencher (TAMRA)—resulting in a relatively poor signal-to-noise ratio. Black Hole Quencher (BHQ™) molecules have been developed to overcome these drawbacks.

Features

- No native fluorescence, resulting in lower background fluorescence
- Increases signal-to-noise ratios, providing higher sensitivity
- Accesses the visible spectrum into near-IR for reporting (480 to 730 nm)
- Maximizes spectral overlap, increasing efficiency of quenching
- Enables wider choice of reporter dyes for multiplexing assays

Absorption Spectra of BHQ dyes among the most commonly used dyes.



BHQ Dye Absorption Maxima and Quenching Range

Quencher	Abs max	Quenching Range (nm)
BHQ-1	534	480 - 580
BHQ-2	579	550 - 650
BHQ-3	672	620 - 730

BHQ Dye / Reporter Combinations

Quencher	Suggested Fluorophores
BHQ-1	FAM, TET, JOE, HEX, Oregon Green®
BHQ-2	TAMRA, ROX, Cy3, Cy3.5, CAL Red™, Red 640
BHQ-3	Cy5, Cy5.5

Proligo Primers & Probes offers a wide range of custom oligonucleotides

Applications & Techniques	Fluorescent Probes	E@sy Oligo™	Fast Oligo™	Guaranteed Oligos™	LNA™ Oligos	Mass Oligos™	siRNA Oligos	WellRED Oligos
PCR ¹		•	•	•	•	•		•
PCR with proofreading enzymes				•	•	•		•
Allele-specific PCR		•	•	•	•	•		•
Real-time quantitative PCR	•	•	•	•	•	•		
Reverse transcription PCR	•	•	•	•	•	•		•
RACE				•	•	•		•
Sequencing		•	•	•	•	•		•
Cloning				•	•	•		•
Primer extension				•	•	•		•
Site-directed mutagenesis				•	•			•
AFLP™ analysis		•	•	•	•	•		•
In situ hybridization				•	•			•
Mutation detection	•			•	•	•		•
Gene silencing	•			•	•	•	•	
Genotyping	•			•	•	•		•
Fragment Analysis	•			•		•		•

For more information on the wide range of custom oligonucleotides offered by Proligo Primers & Probes, please visit our Web site, www.proligo.com or call your local Proligo representative.

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