

Technical Note



Locked Nucleic Acid

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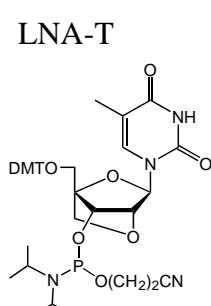
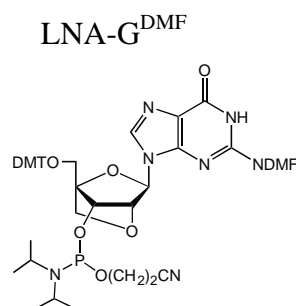
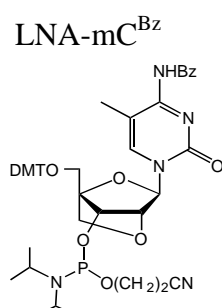
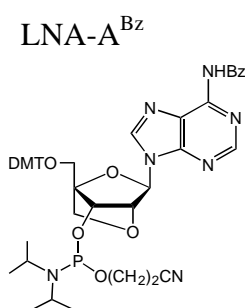
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LNA Oligonucleotide Synthesis

LNA oligonucleotides are synthesised by standard phosphoramidite chemistry using slightly modified protocols. LNA phosphoramidites are supplied with four different nucleobases, Thymine (T), 5-methyl-Cytosine (mC), Adenine (A) and Guanine (G).

Amount of LNA amidite needed for making a 3mL solution of a given concentration.

Amidite	0.07M	0.1M
LNA-A ^{Bz}	186mg	266mg
LNA-T	162mg	232mg
LNA-mC ^{Bz}	184mg	260mg
LNA-G ^{DMF}	179mg	256mg



Synthesis of LNA oligonucleotides

The LNA phosphoramidites are fully compatible with standard DNA synthesis using DNA phosphoramidites and only minor changes in the protocols are recommended in order to get the optimum coupling efficiency of the LNA amidites.

Dissolution of LNA amidites

Except for the LNA-mC amidite, the LNA amidites can be dissolved in standard concentrations using anhydrous acetonitrile. For the LNA-mC amidite it is strongly recommended to use a 25% THF/acetonitrile solution. Use fresh THF to avoid peroxides as they oxidize the phosphoramidite. The amidite should be dissolved in anhydrous THF and diluted to the desired concentration with anhydrous acetonitrile.

To obtain a 3 mL 25% THF/acetonitrile solution, 0.75 mL of THF and 2.25 mL of acetonitrile is needed. Anhydrous dichloromethane can substitute anhydrous THF. The amount of LNA amidites necessary for obtaining 3mL of a 0.07M and a 0.1M solution, respectively, are given in the table.

The amidite should be dissolved in a dry flask (oven at 100°C for at least 3 days and then cooled under inert atmosphere) and should preferably be dissolved 16 hours before use and stand under inert atmosphere at room temperature with molecular sieves added to the solution.

Coupling of LNA amidites

LNA phosphoramidites are more sterically hindered compared to standard DNA phosphoramidites and therefore needs longer coupling time. Normally double coupling time compared to standard DNA phosphoramidites is recommended. Both Tetrazole and Dicyanoimidazole (DCI) works well as activators for the LNA phosphoramidites in oligonucleotide synthesis. Best results are obtained if a 0.5M DCI are used as activator.

Oxidation

The oxidation of the phosphite after the LNA coupling is slower compared to the similar DNA phosphite and longer oxidation time is therefore recommended. A 50 - 100% increase in oxidation contact time compared to standard DNA oxidation contact time is strongly recommended for the oxidation of LNA monomers using standard iodine oxidation procedures.

Deprotection of LNA oligonucleotides

LNA containing oligonucleotides are deprotected following std. protocols. *Note:* It is advised to avoid the use of methylamine when deprotecting oligonucleotides containing LNA-mC since the use of methylamine can result in introduction of an N⁴-methyl modification.

LNA containing oligonucleotides can be purified and analysed using the same methods as for standard DNA oligonucleotides.

Examples

Protocols for LNA couplings on Expedite and ABI3900 DNA-synthesizers can be downloaded at www.exiqon.com

Trademarks and patents

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