

Technical Note



Locked Nucleic Acid

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LNA phosphorothioate synthesis using an Expedite™ DNA synthesizer

The use of LNA phosphoramidites on an Expedite™ instrument to synthesize LNA phosphorothioates follows with minor changes the standard procedures for use of phosphoramidites.

The LNA-A, G and T amidites are used as 0.07M solutions in anhydrous acetonitrile.

For LNA-mC the amidite is first dissolved in anhydrous tetrahydrofurane. When a clear solution has been obtained anhydrous acetonitrile is added to a final THF/ACN ratio of 25:75 and an amidite concentration of 0.07M.

Increased coupling time and sulfur oxidation time is recommended for all LNA phosphoramidites.

We recommend the use of Beaucage's reagent as a 0.2 M (4%) solution:

Dissolve 4g in 100 mL of anhydrous acetonitrile.*

* Do not add sieves as this might promote decomposition of Beaucage's reagent. Use silanized bottles as recommended by the manufacturer.

	Molecular weight g/mole	Product Nr.	CAS. Nr.	Dissolve in	To obtain a 0.07M solution	
					100 mg	250 mg
LNA-A ^{Bz}	885.9	A-0063-	[206055-79-0]	Anhydrous Acetonitrile	1.6 mL	4.1 mL
LNA-mC ^{Bz}	875.9	mC-0066-	[206055-82-5]	THF/Acetonitrile 25/75 (v/v)	1.6 mL	4.1 mL
LNA-G ^{DMF}	852.9	G-0082-	[709641-79-2]	Anhydrous Acetonitrile	1.7 mL	4.2 mL
LNA-T	772.8	T-0064-	[206055-75-6]	Anhydrous Acetonitrile	1.8 mL	4.6 mL

¹ We recommend the use of anhydrous tetrahydrofurane (Aldrich 401757). As an alternative anhydrous dichloromethane (Aldrich 270997) can be used as a substitute for THF.

On the following pages you will find protocols for the synthesis of LNA phosphorothioates using Expedite™ DNA synthesizers with and without MOSS.

Synthesis Cycle for LNA phosphorothioates on an Expedite™ (0.2 μmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```

/*-----*/
/*      Function                Mode  Amount  Time(sec)      Description      */
/*                                     /Arg1   /Arg2                                     */
/*-----*/
$Deblocking
 12 /*Wsh A                    */ PULSE    15     0  "Pre Wsh A"
144 /*Index Fract. Coll.      */ NA       1     0  "Event out ON"
  0 /*Default                  */ WAIT     0    1.5  "Wait"
141 /*Trityl Mon. On/Off     */ NA       1     1  "START data collection"
 16 /*Dblk                     */ PULSE   20     0  "Deblock to column"
 16 /*Dblk                     */ PULSE    2    10  "Deblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 16 /*Dblk                     */ PULSE    2    10  "Slow Debblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 16 /*Dblk                     */ PULSE    2    10  "Slow Debblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 16 /*Dblk                     */ PULSE    2    10  "Slow Debblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 16 /*Dblk                     */ PULSE    2    10  "Slow Debblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 16 /*Dblk                     */ PULSE    2    10  "Slow Debblock"
 16 /*Dblk                     */ PULSE   10     0  "Deblock"
 38 /*Diverted Wsh A          */ PULSE   50     0  "Flush system with Wsh A"
141 /*Trityl Mon. On/Off     */ NA       0     1  "STOP data collection"
144 /*Index Fract. Coll.      */ NA       2     0  "Event out OFF"
$Coupling
  1 /*Wsh                      */ PULSE   15     0  "Flush system with Wsh"
  2 /*Act                      */ PULSE    5     0  "Flush system with Act"
 18 /*A + Act                  */ PULSE    5     0  "Monomer + Act to column"
  2 /*Act                      */ PULSE    3    75  "Couple monomer"
  1 /*Wsh                      */ PULSE    7   175  "Couple monomer"
  1 /*Wsh                      */ PULSE   10     0  "Flush system with Wsh"
$Oxidizing
 17 /*Aux                      */ PULSE   15     0  "SOx to column"
 17 /*Aux                      */ PULSE   15   120  "SOx to column"
 12 /*Wsh A                    */ PULSE   10    60  "Slow pulse to thioate"
 12 /*Wsh A                    */ PULSE   20     0  "Wsh A"
$Capping
 13 /*Caps                     */ PULSE   10     0  "Caps to column"
 13 /*Caps                     */ PULSE   10    30  "Caps to column"
 12 /*Wsh A                    */ PULSE    8    15  "Wsh A"
 12 /*Wsh A                    */ PULSE   30     0  "End of cycle wash"

```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ (1.0 μmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third and fourth line in the coupling step has to be changed, see below.

```

/*-----*/
/*      Function                Mode  Amount  Time(sec)      Description      */
/*                                     /Arg1  /Arg2                                     */
/*-----*/
$Deblocking
 12 /*Wsh A                      */ PULSE    15     0  "Pre Wsh A"
144 /*Index Fract. Coll.         */ NA        1     0  "Event out ON"
   0 /*Default                   */ WAIT        0    1.5  "Wait"
141 /*Trityl Mon. On/Off        */ NA        1     1  "START data collection"
 16 /*Dblk                       */ PULSE    20     0  "Deblock to column"
 16 /*Dblk                       */ PULSE     2    10  "Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 16 /*Dblk                       */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                       */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A            */ PULSE    50     0  "Flush system with Wsh A"
141 /*Trityl Mon. On/Off        */ NA         0     1  "STOP data collection"
144 /*Index Fract. Coll.         */ NA         2     0  "Event out OFF"
$Coupling
  1 /*Wsh                        */ PULSE    15     0  "Flush system with Wsh"
  2 /*Act                        */ PULSE     5     0  "Flush system with Act"
 18 /*A + Act                    */ PULSE     5     0  "LNA-A + Act to column"
 18 /*A + Act                    */ PULSE     2    75  "LNA-A + Act to column"
  2 /*Act                        */ PULSE     3   150  "Couple monomer"
  1 /*Wsh                        */ PULSE     7   200  "Couple monomer"
  1 /*Wsh                        */ PULSE    10     0  "Flush system with Wsh"
$Oxidizing
 17 /*Aux                        */ PULSE    15     0  "SOx to column"
 17 /*Aux                        */ PULSE    15   120  "SOx to column"
 12 /*Wsh A                      */ PULSE    10    60  "Slow pulse to thioate"
 12 /*Wsh A                      */ PULSE    20     0  "Wsh A"
$Capping
 13 /*Caps                       */ PULSE    10     0  "Caps to column"
 13 /*Caps                       */ PULSE    10    30  "Caps to column"
 12 /*Wsh A                      */ PULSE     8    15  "Wsh A"
 12 /*Wsh A                      */ PULSE    30     0  "End of cycle wash"

```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ with MOSS (0.2 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```

/*-----*/
/*      Function                Mode  Amount  Time(sec)      Description      */
/*                                     /Arg1  /Arg2                                     */
/*-----*/
$Deblocking
 12 /*Wsh A                    */ PULSE    15     0  "Pre Wsh A"
144 /*Index Fract. Coll.      */ NA       1     0  "Event out ON"
   0 /*Default                 */ WAIT     0    1.5  "Wait"
 16 /*Dblk                     */ PULSE    20     0  "Deblock to column"
141 /*Trityl Mon. On/Off     */ NA       1     1  "START data collection"
 16 /*Dblk                     */ PULSE    20     0  "Deblock to column"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 38 /*Diverted Wsh A         */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A         */ PULSE     2    10  "Slow Deblock"
 38 /*Diverted Wsh A         */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A         */ PULSE    50     0  "Flush system with Wsh A"
141 /*Trityl Mon. On/Off     */ NA       0     1  "STOP data collection"
144 /*Index Fract. Coll.      */ NA       2     0  "Event out OFF"
$Coupling
   1 /*Wsh                     */ PULSE    10     0  "Flush system with Wsh"
   2 /*Act                     */ PULSE     5     0  "Flush system with Act"
  18 /*A + Act                 */ PULSE     5     0  "Monomer + Act to column"
   2 /*Act                     */ PULSE     5     0  "Chase with Act"
   1 /*Wsh                     */ PULSE    12     0  "Chase with Wsh"
   1 /*Wsh                     */ PULSE    10   250  "Slow pulse to couple"
   1 /*Wsh                     */ PULSE     4     0  "Flush with Wsh"
$Oxidizing
  17 /*Aux                     */ PULSE    30     0  "SOx to column"
  12 /*Wsh A                   */ PULSE    20   180  "Slow pulse to thioate"
  12 /*Wsh A                   */ PULSE    20     0  "Wsh A"
$Capping
  13 /*Caps                    */ PULSE    20     0  "Caps to column"
  12 /*Wsh A                   */ PULSE    20    45  "Wsh A"
  12 /*Wsh A                   */ PULSE    60     0  "End of cycle wash"

```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ with MOSS (1.0 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```

/*-----*/
/*      Function                Mode  Amount  Time(sec)      Description      */
/*                                     /Arg1   /Arg2                                     */
/*-----*/
$Deblocking
 12 /*Wsh A                    */ PULSE    15     0  "Pre Wsh A"
144 /*Index Fract. Coll.      */ NA       1     0  "Event out ON"
   0 /*Default                 */ WAIT     0    1.5 "Wait"
 16 /*Dblk                     */ PULSE    20     0  "Deblock to column"
141 /*Trityl Mon. On/Off     */ NA       1     1  "START data collection"
 16 /*Dblk                     */ PULSE    20     0  "Deblock to column"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 16 /*Dblk                     */ PULSE    10     0  "Deblock"
 16 /*Dblk                     */ PULSE     2    10  "Slow Deblock"
 38 /*Diverted Wsh A          */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A          */ PULSE     2    10  "Slow Deblock"
 38 /*Diverted Wsh A          */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A          */ PULSE    50     0  "Flush system with Wsh A"
141 /*Trityl Mon. On/Off     */ NA       0     1  "STOP data collection"
144 /*Index Fract. Coll.      */ NA       2     0  "Event out OFF"
$Coupling
   1 /*Wsh                     */ PULSE    15     0  "Flush system with Wsh"
   2 /*Act                     */ PULSE     5     0  "Flush system with Act"
  18 /*A + Act                 */ PULSE     7     0  "LNA-A + Act to column"
   2 /*Act                     */ PULSE     5     0  "Couple monomer"
   1 /*Wsh                     */ PULSE    10     0  "Couple monomer"
   1 /*Wsh                     */ PULSE    15    425  "Couple monomer"
   1 /*Wsh                     */ PULSE    20     0  "Flush system with Wsh"
$Oxidizing
  17 /*Aux                     */ PULSE    30     0  "SOx to column"
  12 /*Wsh A                   */ PULSE    20    180  "Slow pulse to thioate"
  12 /*Wsh A                   */ PULSE    20     0  "Wsh A"
$Capping
  13 /*Caps                    */ PULSE    20     0  "Caps to column"
  12 /*Wsh A                   */ PULSE    20    45  "Wsh A"
  12 /*Wsh A                   */ PULSE    60     0  "End of cycle wash"

```

Bottle/position codes:

```

18 /*A + Act                  */
19 /*C + Act                  */
20 /*G + Act                  */
21 /*T + Act                  */
22 /*5 + Act                  */
23 /*6 + Act                  */
24 /*7 + Act                  */
25 /*8 + Act                  */
26 /*9 + Act                  */

```

Trademarks and patents

Exiqon™ is a registered trademark of Exiqon A/S, Vedbaek, Denmark. Locked nucleic acid (LNA™) is covered by patents/ patents applications, and corresponding worldwide applications owned by Exiqon A/S and Prof. Imanishi. Expedite™ is a trademark of Applied Biosystems.

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