

Technical Note



Locked Nucleic Acid

LNA14/05.2005

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LNA Oligonucleotide Synthesis on Expedite™ Multiple Oligo Synthesis System (MOSS)

The use of LNA phosphoramidites on an Expedite™ instrument follows standard procedures for use of phosphoramidites.

The LNA-A, G and T amidites are used as 0.07M solutions in anhydrous acetonitrile, in the case of LNA-mC it is advised to dissolve the amidite in a 25% tetrahydrofurane/acetonitrile¹ solution (0.07M) to avoid precipitation of the amidite.

A 250 seconds coupling time is normally found to be sufficient for all LNA phosphoramidites.

It is recommended to use 0.5M dicyanoimidazole (DCI) as activator².

LNA requires a longer oxidation time compared to DNA 45 seconds has been found optimal when synthesising standard oligonucleotides.

	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.

² A 0.5M DCI solution is not commercial available. A 0.5M DCI solution can be made by dissolving 5.9g of DCI (Aldrich 67864) in 100mL of anhydrous acetonitrile.

Synthesis Cycle for LNA on an Expedite™ Multiple Oligo Synthesis System (MOSS)

The synthesis cycle has been written for position 5 on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

MOSS LNA-cycle (0.2µmole scale)

```

/* ----- */
/*      Function              Mode  Amount  Time(sec)      Description      */
/*              /Arg1      /Arg2                                     */
/* ----- */
$Deblocking
 12 /*Wsh A                  */ PULSE    15     0  "Pre cycle wash"
144 /*Index Fract. Coll.    */ NA        1     0  "Event out ON"
   0 /*Default              */ WAIT     0    1.5 "Wait"
 16 /*Dblk                  */ PULSE    20     0  "Dblk to column"
141 /*Trityl Mon. On/Off    */ NA        1     1  "START data collection"
 16 /*Dblk                  */ PULSE    20     0  "Deblock"
 16 /*Dblk                  */ PULSE     5    10  "Deblock"
 16 /*Dblk                  */ PULSE    10     0  "Deblock"
 16 /*Dblk                  */ PULSE     5    10  "Deblock"
 16 /*Dblk                  */ PULSE    10     0  "Deblock"
 16 /*Dblk                  */ PULSE     5    10  "Deblock"
 16 /*Dblk                  */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A        */ PULSE    20    20  "Deblock"
 38 /*Diverted Wsh A        */ PULSE    40     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"
 22 /*5 + 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"
$Capping
 13 /*Caps                  */ PULSE    12     0  "Caps to column"
 12 /*Wsh A                 */ PULSE    10     0  "Chase with Wsh A"
 12 /*Wsh A                 */ PULSE    10    45  "Slow pulse to cap"
$Oxidizing
 15 /*Ox                   */ PULSE    15     0  "Ox to column"
 12 /*Wsh A                 */ PULSE    12     0  "Chase with Wsh A"
 12 /*Wsh A                 */ PULSE    10    45  "Slow pulse to Ox"
$Capping
 13 /*Caps                  */ PULSE    10     0  "Caps to column"
 12 /*Wsh A                 */ PULSE    10     0  "Chase with Wsh A"
 12 /*Wsh A                 */ PULSE    10    10  "Slow pulse to Cap"
 12 /*Wsh A                 */ PULSE    20     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              */

```

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.

MOSS DNA-cycle (0.2µmole scale)

```

/* ----- */
/*      Function           Mode  Amount  Time(sec)      Description      */
/*                               /Arg1  /Arg2                               */
/* ----- */
$Deblocking
 12 /*Wsh A                */ PULSE    15     0  "Pre cycle wash"
144 /*Index Fract. Coll.   */ NA         1     0  "Event out ON"
  0 /*Default              */ WAIT         0    1.5 "Wait"
 16 /*Dblk                 */ PULSE    20     0  "Dblk to column"
141 /*Trityl Mon. On/Off   */ NA         1     1  "START data collection"
 16 /*Dblk                 */ PULSE    20     0  "Deblock"
 16 /*Dblk                 */ PULSE     5    10  "Deblock"
 16 /*Dblk                 */ PULSE    10     0  "Deblock"
 16 /*Dblk                 */ PULSE     5    10  "Deblock"
 16 /*Dblk                 */ PULSE    10     0  "Deblock"
 16 /*Dblk                 */ PULSE     5    10  "Deblock"
 16 /*Dblk                 */ PULSE    10     0  "Deblock"
 38 /*Diverted Wsh A       */ PULSE    20    20  "Deblock"
 38 /*Diverted Wsh A       */ PULSE    40     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    120  "Slow pulse to couple"
  1 /*Wsh                  */ PULSE     4     0  "Flush with Wsh"
$Capping
 13 /*Caps                 */ PULSE    12     0  "Caps to column"
 12 /*Wsh A                */ PULSE    10     0  "Chase with Wsh A"
 12 /*Wsh A                */ PULSE    10    45  "Slow pulse to cap"
$Oxidizing
 15 /*Ox                   */ PULSE    15     0  "Ox to column"
 12 /*Wsh A                */ PULSE    12     0  "Chase with Wsh A"
 12 /*Wsh A                */ PULSE    10    30  "Slow pulse to Ox"
$Capping
 13 /*Caps                 */ PULSE    10     0  "Caps to column"
 12 /*Wsh A                */ PULSE    10     0  "Chase with Wsh A"
 12 /*Wsh A                */ PULSE    10    10  "Slow pulse to Cap"
 12 /*Wsh A                */ PULSE    20     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              */

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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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