NittoPhase®HL Solid Support

High Loaded Polymeric Solid Supports for Oligonucleotide Synthesis

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Introduction

Increasing interest in the development of oligonucleotide therapeutic molecules, especially RNAi, has led to a growing need to optimize synthesis. Materials with higher performance to price ratio will allow drug development companies to meet their API cost targets. Solid support is one component that has come under close scrutiny in this regard. Controlled Pore Glass (CPG) supports have a limited loading capacity of around 80-90 µmol/g, while polymeric supports, capable of achieving significantly higher loading, have historically failed to show satisfactory and reliable performance at loadings greater than 200 µmol/g for DNA and 150 µmol/g for RNA synthesis. To address this issue, we have developed NittoPhase®HL, a high performance solid support with optimized loading capacities of up to 250 µmol/g and 400 µmol/g for RNA & DNA oligonucleotide syntheses, respectively. Since its development in 2010, NittoPhase®HL has shown superb performance in both synthesis yields and purities as compared to other solid supports in side-by-side comparisons. Regardless whether it is a 1-2 mmol lab scale or clinical trial material production batch (at 220, 550, 700 and 900 mmol scales), NittoPhase®HL has always produced highly favorable results in DNA synthesis. In these studies, NittoPhase®HL loaded with UnyLinker™ at 350 µmol/g was utilized for synthesis of 20-mer MOE Gapmers and achieved per micromole yields (OD_{260}/µmol) higher or equivalent to alternative supports. Meanwhile, NittoPhase®HL at loading of 250 µmol/g has also been successfully utilized in syntheses of highly modified siRNAs in various scales (ranging from 65 µmol to 65 mmol) to provide prominent crude yields and purities. These increased yields and purities, together with the elevated loading capacities, undoubtedly empower NittoPhase®HL to offer superior performance at lower unit cost for oligonucleotide synthesis.

DNA Synthesis Results*

Sequence	Synthesis Scale (mmol)	Support Type	Loading (µmol/g)	Crude Purity (%)	Crude Yield (OD_{260}/µmol)
5-10-5 MOE gapmer	1	Support A	360	78.5	93
				78.6	93
5-10-5 MOE gapmer	1	NittoPhase®HL	431	68.8	80
5-10-5 MOE gapmer	2	NittoPhase®HL	350	80.4	101
				75.2	94
5-10-5 MOE gapmer	2	NittoPhase®HL	400	71.4	90
				72.8	93
3-10-3 MOE gapmer	2	Support A	360	82.8	100
				83.1	100
3-10-3 MOE gapmer	2	NittoPhase®HL	324	82.8	101
				83.0	105
5-10-5 MOE gapmer	220	Support A	316	81.0	108
5-10-5 MOE gapmer	220	Support A	325	79.6	100
5-10-5 MOE gapmer	220	NittoPhase®HL	319	83.2	108
5-10-5 MOE gapmer	550	NittoPhase®HL	319	86.3	111
5-10-5 MOE gapmer	700	NittoPhase®HL	315	90.7	112
5-10-5 MOE gapmer	900	NittoPhase®HL	315	83.2	105

* Data courtesy of Isis Pharmaceuticals, Inc.

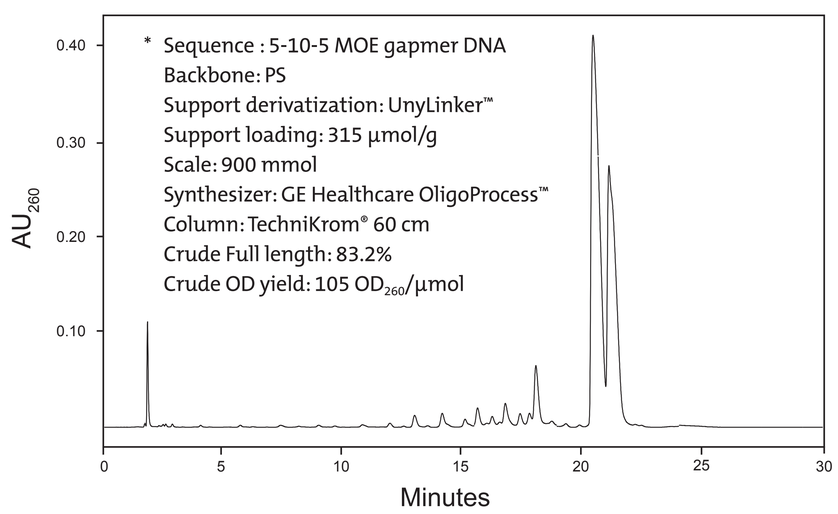

Figure 1. IP-HPLC-UV Trace for NittoPhase®HL UnyLinker™ DNA synthesis.

Standard RNA Synthesis Results

Sequence	Synthesis Scale	Support Type	Loading (µmol/g)	Weight Yield (mg/µmol)	Crude Purity (%)	Crude yield (OD_{260}/µmol)
5-5-5-5-1-mer	200 µmol	Support A	308	5.06	71.2	141
5-5-5-5-1-mer	175 µmol	NittoPhase®HL	251	6.22	75.4	175
3-5-5-6-2-mer	303 µmol	NittoPhase®HL	244	8.76	74.0	124
6-5-5-3-2-mer	567 µmol	NittoPhase®HL	207	9.49	84.8	112
4-2-7-6-2-mer	1 mmol	NittoPhase®HL	253	7.27	62.4	87
4-2-7-6-2-mer	4 mmol	NittoPhase®HL	253	8.56	77.4	120

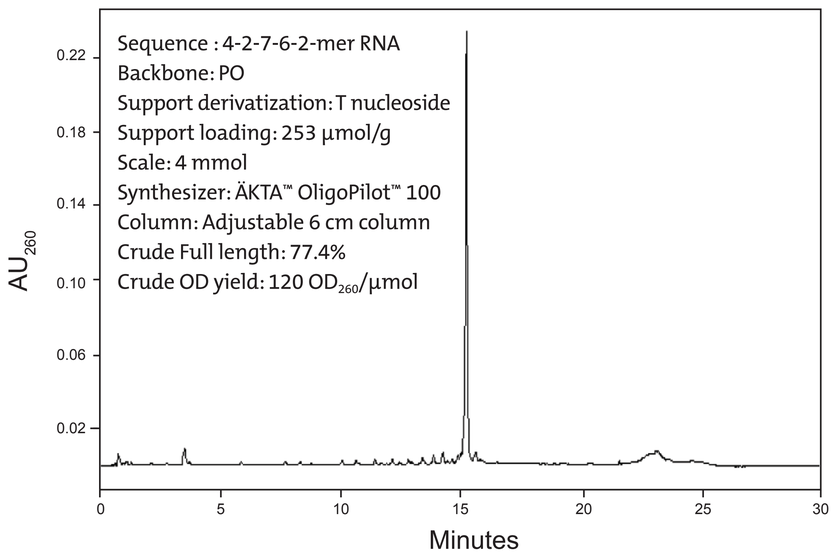

Figure 2. IP-HPLC-UV Trace for NittoPhase®HL T250 RNA synthesis.

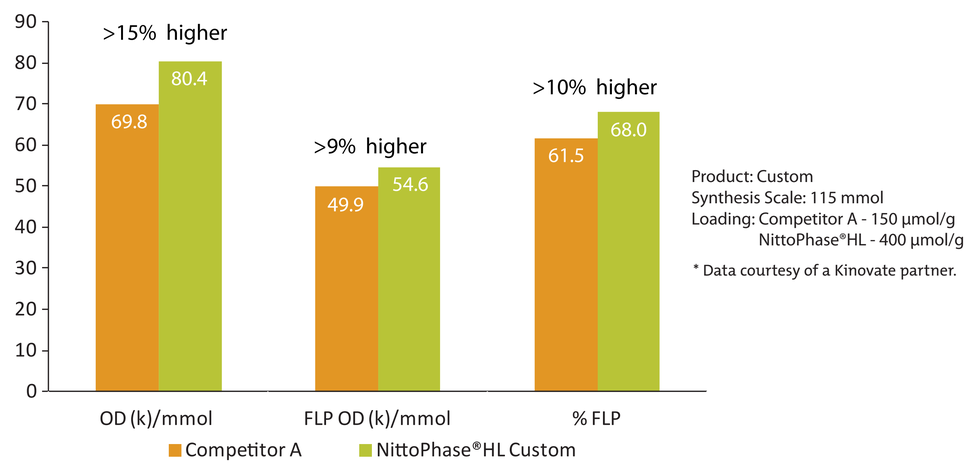

Figure 3. Custom loaded solid support performance comparison.

Highly Modified RNA Synthesis Results

Suppor Type	Loading (µmol/g)	Synthesis Scale	Strand	Crude Purity (%)	Crude Yield (OD_{260}/µmol)
Support C	30	65 µmol	Sense	61	122
			Antisense	58	78
NittoPhase®HL	250	325 µmol	Sense	74	128
			Antisense	68	81
NittoPhase®HL	250	2 mmol	Sense	79	145
			Antisense	84	142
NittoPhase®HL	250	16.4 mmol**	Sense	73	149
			Antisense	78	157
NittoPhase®HL	250	65 mmol**	Sense	76	151
			Antisense	79	136

** In collaboration with NITTO DENKO Avecia Inc. & Quark Pharmaceuticals, Inc.

Polymer Support Properties

Product Properties		NittoPhase®	NittoPhase®HL
Functionality		Hydroxyl group	Hydroxyl group
# of Hydroxyl Group		420 µmol/g	550 µmol/g
Average Particle Size		90 µm	85 µm
Average Pore Size		55 nm	45 nm
Dry Volume		3.2 ml/g	2.7 ml/g
Optimal loading		200 µmol/g	200 – 400 µmol/g
Swelling Volume (in ACN)		3.85 ml/g	4.0 ml/g
Swelling Volume (in toluene)		6.4 ml/g	6.1 ml/g
Column Packing Recommendation (6.3 ml Column)	DNA	1.10 g (20-mer, 200 µmol/g)	0.80 g (20-mer, 350 µmol/g)
	RNA	1.05 g (21-mer, 150 µmol/g)	0.69 g (21-mer, 250 µmol/g)

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