A

Oligonucleotide Synthesis

DNA Oligonucleotide
RNA Oligonucleotide

DNA Oligonucleotide
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01

DNA Oligonucleotide *AccuOligo*®

DNA Oligonucleotide (*AccuOligo*®)

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Overview

High quality oligos and reasonable price.

Bioneer, founded in 1992, is one of leading suppliers of synthetic oligonucleotides in the world (DNA and RNA). In-house production of raw materials (phosphoramidites, reagents & solvents) along with automated oligo synthesis and purification systems result in superior quality oligonucleotides at a reasonable price.

Since Bioneer manufactures all the components of our oligonucleotides, we manage the quality control every step of the way – ensuring that you receive only the highest quality product. Bioneer's oligonucleotides are purified through our unique Bio-RP cartridge purification technology. Bio-RP removes many impurities and synthesis failure products that are still present in an oligo after desalting*. These impurities contribute to the OD measurement of competitor oligonucleotides that are merely desalted – and artificially inflate their yield (Figure 1.). The end result of Bioneer's Bio-RP cartridge purification is near HPLC quality purification with only full-length product. We are so confident in our oligonucleotides that we are the only company to offer a 100 % guarantee that they will work for your PCR or qPCR application!

With high throughput oligo synthesis facilities around the world, Bioneer's daily capacity is unsurpassed. Bioneer is unrivalled in its ability to address the needs of customers requiring a few oligonucleotides on a regular basis and those customers that require very large numbers of oligonucleotides on a less frequent basis. We respond to your needs personally.

Why is Bio-RP purification better than desalting?

Most oligos in the market are merely deprotected and desalted. The process of deprotection/desalting only partially purifies the oligo and leaves behind many impurities* and failure products (truncated oligos that will not amplify, and can even interfere with certain applications). These can lead to artificially inflated OD readings that increase with oligo length. Bio-RP purification removes all these contaminants and failure products, resulting in an oligo that is near HPLC purity. The advantage to you is that our product contains only quality oligos without impurities that can inhibit some PCR reactions, and potentially skew qPCR reactions. We have the same amount of active oligo as our competitors; they just have more "Other" inflating their OD. To demonstrate this, plates of oligos of various sizes were tested for concentration in 2 steps:1) After a deprotection / desalting step only, and 2) after Bio-RP purification(Figure 1).

The results of these tests show that the amount of failed sequences and impurities* increase according to oligo length, but are not removed by deprotection/desalting process. This results in inflated OD readings for oligos depend on oligo size (Table 1).

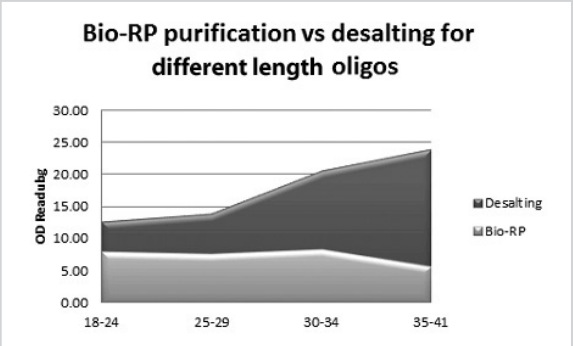

Figure 1. Plates of oligos of various lengths were tested for concentration after desalting and then by Bio-RP cartridge purification. Note the inflated OD readings when deprotection / desalting alone is used.

Oligo Size	18 - 24	24 - 29	30 - 34	35 - 41
OD Inflation	38 %	45 %	60 %	77 %

Table 1. Average OD inflation seen for oligos of various sizes when protection/desalting alone are employed. Note for longer oligos, up to 77% of product does not consist of functional oligo.

* Impurities in desalted oligos may include: Acetonitrile, Pyridine, Iodine, Ethyl thio tetrazole, Dichloromethane, Acetic acid, Acrylonitrile, Benzamide and Isobutyramide.

01
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***AccuOligo*® is Bioneer's patented oligo technology.**

AccuOligo® prevents oligo dislodging and potential loss during packing, shipping and opening, by the addition of an adhesive in the tube during the production process. Even the 96-deep well plate format orders have *AccuOligo*® technology applied to prevent possible cross-contamination (Dried oligonucleotide composition of the patent. Registration Number 10-0777249)

- The adhesive does not affect PCR, Sequencing, restriction digests or other experimental methods.
- Prevention of dislodging and loss of oligos during production, packaging and shipping
- Prevention of loss by oligo "flaking" and tube cup attachment.
- Prevention of cross-contamination during product of 96 well plate format orders

- **Bioneer's in-house developed Bio-RP purification system.**
  Bioneer has developed RP resin that tightly binds synthesized oligos and applied the resin to the Bio-RP purification system, allowing us to provide high-purity oligos to our customers.

- **Contamination prevention through clean room production.**
  All oligos manufactured in a clean room facility, allowing us to provide nuclease / nucleic acid-free products to our customers.

- **Molecular weight-level quality control with accurate MALDI- TOF QC.**
  Bioneer provides the highest quality products through strict Q.C systems with MALDI-TOF and 96 well CE.

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DNA Oligonucleotide (AccuOligo®)

Standard Oligonucleotide

Description

For routine applications, Bioneer offers standard custom oligos with Bio-RP purification, provided in a tube format. Please contact your sales representative to learn futher information about any institutional or volume discounts we might have in place for our oligonucleotides.

Features and Benefits

- Free Bio-RP purification: Near HPLC purity at a standard oligo price
- Quick turnaround time: 24 - 48 hours from order to bench in most cases
- Broad range of modifications available: If there is no modification listed that you want, you can ask it to your sales representative.
- Competitive pricing: Great value for your research dollar

Additional Service

Post-Handling
Concentration normalized - Standard
Concentration normalized - Custom
Bar-code Label
Dried
Aliquoting to daughter Plates
Mixing primers
Individual Tube plate-Labeled
Custom plate
Double strand (Annealing service)
MALDI-TOF (QC)
MALDI-TOF (SNP or Genotyping)
Shipping Charge
Hand mix service (MIX base Code)
Remainder in plate service
Circular ssDNA service

Custom Analytical Service

Items
I-E HPLC
RP HPLC
Capillary electrophoresis
PAGE analysis
MALDI-TOF analysis, Spectrocheck
MALDI-TOF analysis, other mass system

Guaranteed Yield and Delivery Time

Synthesis Scale (μmole)	Base Limitation (mer)	Guaranteed Yield (OD)			Delivery Time (Days)		
		Bio-RP	PAGE	HPLC	Bio-RP	PAGE	HPLC
0.025	15 - 60	2	1	1.5	2	3	3
0.05	10 - 75	4	2	2.5	2	3	3
0.2	5 - 110	8	6	7	2	3	3
1	5 - 130	30	18	25	2	3	3
10	5 - 50	300	150	200	2	3	3
15	5 - 50	Inquire	Inquire	Inquire	3	4	4

* The synthesis scales refer to the initial starting point for a synthesis. The starting synthesis scale and the length of the oligo will influence the final yield.

01
DNA Oligonucleotide (AccuOligo®)

HT-Oligo™ (High Throughput Oligonucleotide)

■ Description

A total capacity of 30,000 oligonucleotides per day gives Bioneer the opportunity to address the needs of researchers who require gram scale of oligonucleotides. In-house production of raw materials along with proprietary high throughput oligo synthesis and purification systems result in much higher quality oligonucleotides at significantly lower cost. Bioneer understands that high quality and low cost is especially important to our high throughput screening oligo users.

■ Features and Benefits

- Free Bio-RP purification: Near HPLC purity at a standard oligo price
- Quick turnaround time: 48 hours from order to bench in most cases
- Broad range of modifications available:
- Competitive pricing: Great value for your research dollar

■ Guaranteed Yield and Delivery Time

Synthesis Scale (μmole)	Base Limitation (mer)	Guaranteed Yield (OD)			Delivery Time (Days)		
		Bio-RP	PAGE	HPLC	Bio-RP	PAGE	HPLC
0.025	15 - 60	2	1	1.5	2	3	3
0.05	10 - 75	4	2	2.5	2	3	3
0.2	5 - 110	8	6	7	2	3	3
1	5 - 130	30	18	25	2	3	3
10	5 - 50	300	150	200	Inquire	Inquire	Inquire
15	5 - 50	Inquire	Inquire	Inquire	Inquire	Inquire	Inquire

* The synthesis scales refer to the initial starting point for a synthesis. The starting synthesis scale and the length of the oligo will influence the final yield.

Modification Oligonucleotide

Description

Bioneer provides the ideal tools for your specialized applications. With the advent of technologies such as Real-Time detection and quantification, as well as mass-scale genotyping projects, more and more scientists require the use of modified oligos. All common oligo modifications are available. Our fluorogenic oligos for qPCR, are unsurpassed in quality, and priced for value. Please contact oligo-support@bioneer.com for more information on our modified oligos.

Features and Benefits

- Free Bio-RP purification: Near HPLC purity at a standard oligo price
- Quick turnaround time: 3 - 4 days from order to bench in most cases
- Broad range of modifications available:
- Competitive pricing: Great value for your research dollar

Modification Oligos

3' Modification

Modification		
DIG`	2′,3′-ddC	3' AMCA (amino-methyl-coumarin-acetate)
C6 Amine	AlexaFluor 488	3' Deoxypurine (2'-DeoxyNebu larine)
Phosphate	AlexaFluor 532	3' PEG-2000
Biotin	AlexaFluor 546	3' Azide
C3 spacer	AlexaFluor 594	3' Acrylamide (acrydite)
C6 spacer	AlexaFluor 647	O6-Methyl 2'-dG
C12 spacer	AlexaFluor 660	O4-Methyl-dT
C18 atom spacer	AlexaFluor 750	dT-Alkyne
dS spacer	N6-methyl-2′-deoxyadenosine	Rhodamine 6G
FAM	DNP(2,4-dinitrophenyl)-TEG	Epoch Eclips Quencher
TAMRA	Cy3.5	
Thiol	Cy5.5	
Texas Red	Puromycine	
JOE	Yakima Yellow	
ROX	3′-dA	
Cy5	3′-dC	
Cy3	3′-dG	
Dabcyl	3′-dT	
BHQ1	5-Nitroindole	
BHQ2	2′-F-rA	
Cholesteryl	2′-F-rC	
3′-Inverted dT	2′-F-rG	
3′-Inverted dA	2′-F-rU	
3′-Inverted dC	Maleimide	
3′-Inverted dG	Thymidine Glycol	
Ara-dC	Zebularine	
5-F-dU	3' Methylene Blue	

01 DNA Oligonucleotide (AccuOligo®)

Modification Oligonucleotide

5′ Modification

Modification		
DIG	5-F-dU	2-Aminopurine
C6 Amine	2′,3′-ddA	2,6-Diaminopurine
Phosphate	2′,3′-ddC	Dithiol
Biotin	2′,3′-ddG	BHQ2
Thiol	2′,3′-ddT	EDTA-C2-dT
C3 spacer	AlexaFluor 488	Thymidine Glycol
C6 spacer	AlexaFluor 532	Zebularine
C12 spacer	AlexaFluor 546	5' Methylene Blue
18 atom spacer	AlexaFluor 594	5' AMCA (amino-methyl-coumarin-acetate)
dS spacer	AlexaFluor 647	5' Carboxy-dT
FAM	AlexaFluor 660	5' Deoxypurine (2'-DeoxyNebu larine)
TAMRA	AlexaFluor 750	5' PEG-2000
HEX	N6-methyl-2′-deoxyadenosine	5'-BromoHexyl (Br)
TET	PC(photo-cleavable) Amine Linker	5' Acridine
Texas Red	PC(photo-cleavable) Biotin Linker	5' Acrylamide (acrydite)
JOE	DNP(2,4-dinitrophenyl)-TEG	5'-Yakima Yellow
ROX	Cy3.5	O6-Methyl 2'-dG
Cy5	3′-dA	O4-Methyl-dT
Cy3	3′-dC	5' C3-Amine
IRD700	3′-dG	5' C12-Amine
IRD800	3′-dT	dT-Alkyne
C10 Carboxylic Acid	5-Nitroindole	Rhodamine 6G
C2 Aldehyde	2′-F-rA	Pyrene-Cap
Cy5.5	2′-F-rC	Epoch Eclips Quencher
Cholesteryl	2′-F-rG	5'-Hexynyl
Dabcyl	2′-F-rU	
Ara-dC	Maleimide	

Modification Oligonucleotide

Internal Modification

Modification		
Internal Amino Modifier C6 dT	Cy3.5	Deoxypurine (2'-DeoxyNebu larine)
Internal Biotin-dT	Cy5.5	Azide
Fluorescein dT	Puromycine	Trebler Branching
C3 spacer	Yakima Yellow	O6-Methyl 2'-dG
C6 spacer	3′-dA	O4-Methyl-dT
C12 spacer	3′-dC	dT-Alkyne
18 atom spacer	3′-dG	Epoch Eclips Quencher
dS spacer	3′-dT	Thiol-dT
Phosphorothioate (per insertion)	5′-Nitroindole	Cy3 dA
5-Methyl dC	2′-F-rA	Cy3 dC
Inosine	2′-F-rC	Cy3 dG
Deoxy Uridine	2′-F-rG	Cy3 dT
2'-O-Methyl	2′-F-rU	Cy5 dA
5-Bromo dU	2-Aminopurine	Cy5 dC
8-Oxo-dA	2,6-Diaminopurine	Cy5 dG
8-Oxo-dG	Dithiol	Cy5 dT
Ferrocene-dT	EDTA-C2-dT	
5-hydroxymethyl-dC	Thymidine Glycol	
5-Hydroxy-dU	Zebularine	
5-hydroxymethyl-dU	Biotin-TEG	
Ara-dC	HEX-dT	
5-F-dU	Methylene Blue	
N6-methyl-2′-deoxyadenosine	Carboxy-dT	
PC(photo-cleavable)Linker	Tamra-dT	
DNP(2,4-dinitrophenyl)-TEG	BHQ1-dT	
Cy3	BHQ2-dT	
Cy5	Dabcyl-dT	

01
DNA Oligonucleotide (AccuOligo®)

Dual-Labeled Probes

Description

Dual-labeled probes, widely-used for Real-Time qPCR, normally have a reporter dye at the 5′ end and a quencher at the 3′ end. Probes can be used for the sensitive quantitative or qualitative detection of genes. By attaching different types of fluorophores, multiplex reactions to analyze multiple genes are possible. For quality assurance, Bioneer's dual-labeled probes are quality controlled through MALDI-TOF mass analysis, and can be lower in cost and faster in delivery compared to competitor offers.

Features and Benefits

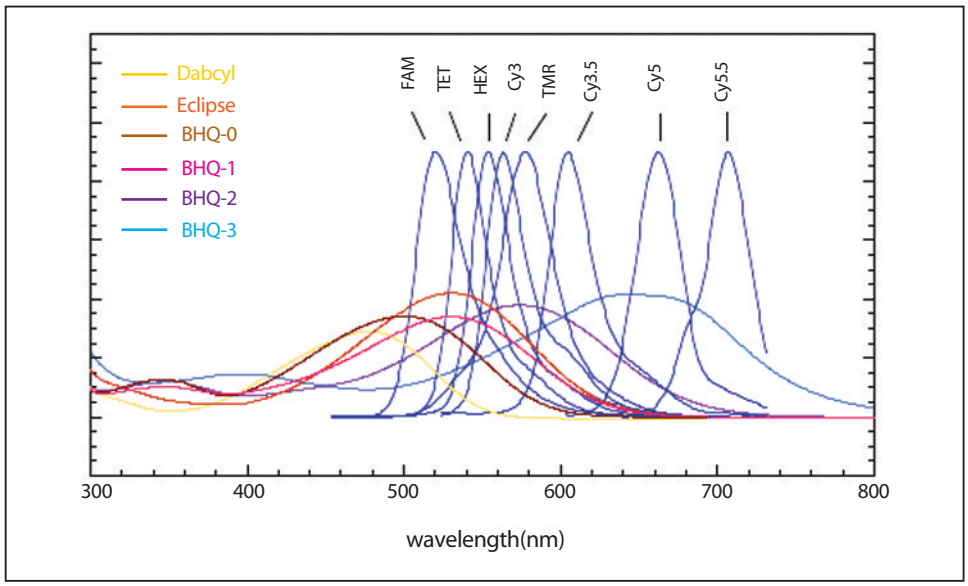
- Quick turnaround time: 4 - 5 days from order to bench in most cases
- Broad range of modifications available: If you don't see it, just ask, we can probably do it.
- Competitive pricing: Great value for your research dollar

Spectra of Fluorescent Dyes

Dye	Excitation max (nm)	Exitinction Coefficient (L/mole.Cm)	Emission max (nm)
6-FAM	494	83,000	520
Fluorescein-dT	494	83,000	522
TET	521	73,000	541
HEX	535	73,000	553
TAMRA	556	91,000	580
Cy3	546	150,000	563
Cy3.5	581	150,000	596
Cy5	646	250,000	662
Cy5.5	675	250,000	694
Cy7	743	250,000	767
JOE	529	71,000	555
ROX	588	82,000	608
Texas Red	598	116,000	617
NED	546	-	575
VIC	538	-	554
IR700	685	170,000	705
IR800	787	200,000	807
Rhodamine 6G	524	116,000	550
DABCYL	478	32,000	-
BHQ-1	534	34,000	-
BHQ-2	579	38,000	-

* (Reference) UV/Visible spectra of the variety of dyes and quenchers currently available.

Dual-Labeled Probes



Combination of Dual-Labeled Probes

Dye	Excitation max (nm)	Emission max (nm)	Compatible Quencher			
			Dabcyl	tamra	BHQ1	BHQ2
6-FAM	494	520				
JOE	529	555				
TET	521	541				
HEX	535	553				
VIC	538	554				
Cy3	546	563				
NED	546	575				
TAMRA	556	580				
Cy3.5	581	596				
ROX	588	608				
Texas Red	598	617				
Cy5	646	662				
Cy5.5	675	694				
IR700	685	705				
Cy7	743	767				
IR800	787	807				
DABCYL	478	-				
BHQ-1	534	-				
BHQ-2	579	-				

01
DNA Oligonucleotide (AccuOligo®)

Dual-Labeled Probes

■ Dual-labeled Probes

Modification		
5'-FAM-3'-TAMRA	5'-HEX-3'-BHQ1	5'-JOE-3'-BHQ2
5'-HEX-3'-TAMRA	5'-TET-3'-BHQ1	5'-TAMRA-3'-BHQ2
5'-TET-3'-TAMRA	5'-JOE-3'-BHQ1	5'-ROX-3'-BHQ2
5'-JOE-3'-TAMRA	5'-TAMRA-3'-BHQ1	5'-Texas Red-3'-BHQ2
5'-FAM-3'-DABCYL	5'-ROX-3'-BHQ1	5'-Cy5-3'-BHQ2
5'-HEX-3'-DABCYL	5'-Texas Red-3'-BHQ1	5'-Cy3-3'-BHQ2
5'-TET-3'-DABCYL	5'-Cy3-3'-BHQ1	5'-FAM-BHQ1-dT-Amine-3'
5'-TAMRA-3'-DABCYL	5'-FAM-3'-BHQ2	5'-FAM-Tamra-dT-PO4-3'
5'-JOE-3'-DABCYL	5'-HEX-3'-BHQ2	
5'-FAM-3'-BHQ1	5'-TET-3'-BHQ2	

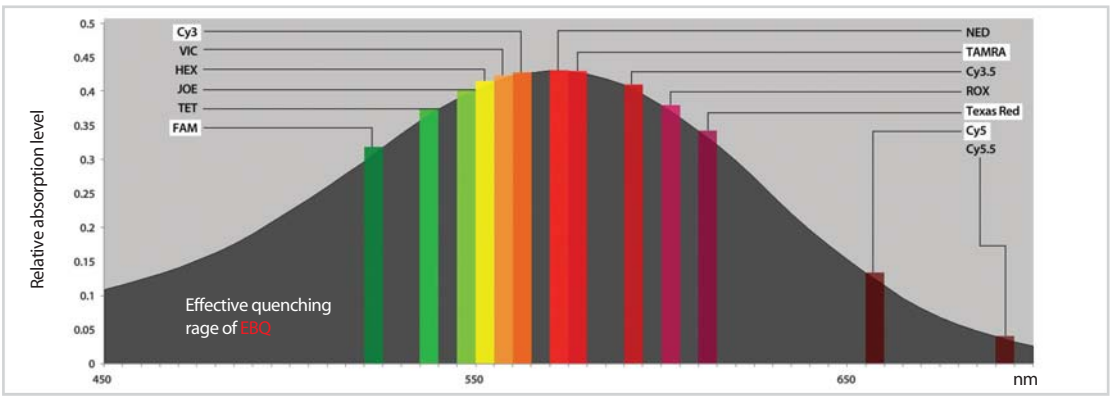
* Please enquire Bioneer's technical staff for Dual-labeled Probe that are not listed above.

Unify your Quencher into Bioneer's EBQ

Description

Fluorescence spectroscopy is a valuable tool for detecting fluorescence levels of biological molecules with fluorophores (dyes) such as Marina Blue, Pacific Blue, Oregon Greens, CyTMdyes, AMCA, Bodipy derivatives, Fluorescein derivatives, FAM, JOE, TET, HEX, VIC, Cy3, NED, TAMRA, Cy3.5, ROX, Texas Red, Cy5, Cy5.5 Quenchers are generally used for quenching fluorescent signals from the fluorescent dyes. These quenching effects have been widely using for detecting the level of the molecule which is of interest in cells or in samples. Previously, BHQ quenchers (BHQ1, BHQ2) were commonly used because they have a high potency of quenching activity. However BHQ1 and BHQ2 are only active in a narrow range of wavelength (BHQ1; ca 400-570 nm, BHQ2; ca 560-700 nm). In order to overcome the limits of BHQs, Bioneer has developed a new molecule EBQ(Excellent Bioneer Quencher), which has better quenching effects in wider range of wavelength (400-700 nm) than BHQs at an affordable price. Now experience the benefits of EBQ; wide wavelength coverage, superior quality, and less cost for your fluorescence spectroscopy experiment!

Figure 1. EBQ effectively quenched most commonly used reporter-dyes (Absorption spectrum of EBQ with the emission spectra of reporter-dyes).



Tab 1. Quenching rage of EBQ compared to other quenchers

Dye	Excitation Max(nm)	Emission Max(nm)	Compatible Quencher				
			Dabcyl	Tamra	BHQ1	BHQ2	EBQ
6-FAM	494	520					
JOE	529	555					
TET	521	541					
HEX	535	553					
VIC	538	554					
Cy3	546	563					
NED	546	575					
TAMRA	556	580					
Cy3.5	581	596					
ROX	588	608					
Texas Red	598	617					
Cy5	646	662					
Cy5.5	675	694					
IR700	685	705					
Cy7	743	767					
IR800	787	807					

01 DNA Oligonucleotide (AccuOligo®)

Unify your Quencher into Bioneer's EBQ

Tab 2. EBQ Dual-Labeled Probes

Modification
5′-FAM-3′-EBQ
5′-HEX-3′-EBQ
5′-TET-3′-EBQ
5′-JOE-3′-EBQ
5′-TAMRA-3′-EBQ
5′-ROX-3′-EBQ
5′-Texas Red-3′-EBQ
5′-Cy5-3′-EBQ
5′-Cy3-3′-EBQ
Most commonly used reporter dyes - EBQ

Features and Benefits

- **Broad- range of wavelength coverage**: A wide absorbance range 400 ~ 700 nm for effective quenching of emission signal from dyes (Maximum absorption occurred at 570 nm). You can unify quenchers into EBQ.
- **Stability**: Structurally stable as a quencher against the change in temperature or pH
- **Variety option of selectable dyes**: EBQ effectively quenches most commonly used reporter-dyes with emission of 400-700 nm wavelength range such as Marina Blue, Pacific Blue, Oregon Greens, CyTMdyes, AMCA, Bodipy derivatives, Fluorescein derivatives, FAM, JOE, TET, HEX, VIC, Cy3, NED, TAMRA, Cy3.5, ROX, Texas Red, Cy5, Cy5.5.
- **Fast and reliable Customer Service**
  Quick turnaround time: 4-5days from order to bench in most cases
- **Competitive pricing**: Great value for your research dollar

Application

Real-Time RT qPCR, Molecular diagnostics, in situ hybridization, etc.

Technical/Specs

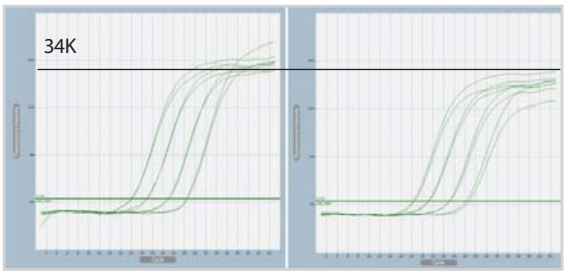
Tab 3. and Fig. 2. EBQ more efficiently quenched FAM dye compared to BHQ1

1) Quenching efficiency measurement through S1 nuclease (Tab. 3)

Dual-Labeled Probe	Fluorescence Signal Intensity			Efficiency (%)
	Before	After	Difference	
EBQ-FAM	4.41	93.17	88.76	113.0
BHQ1-FAM	4.20	82.74	78.54	100.0

FAM-Excitation: 494 nm, Emission: 520 nm

2) Quenching efficiency measurement through △Rn measurement (Figure 2.)



*EBQ *BHQ1

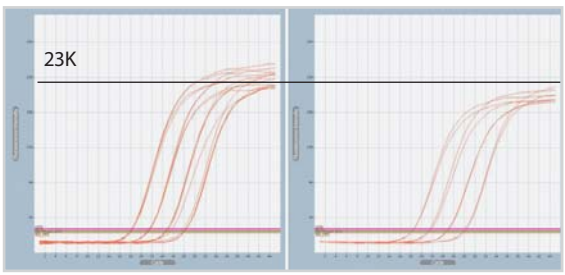
Tab 4 and Figure 3 . Comparison of quenching effect of EBQ-ROX and BHQ-ROX probes

1) Quenching efficiency measurement through S1 nuclease (Tab. 4)

Dual-Labeled Probe	Fluorescence Signal Intensity			Efficiency (%)
	Before	After	Difference	
EBQ-ROX	1.89	65.25	63.37	124.7
BHQ2-ROX	1.14	51.94	50.80	100.0

ROX-Excitation: 588 nm, Emission: 608 nm

2) Quenching efficiency measurement through △Rn measurement (Figure 3.)



*EBQ *BHQ1

*This product is covered by patent(s) (pending) applied by Bioneer Corporation.
Please contact us for further information on commercial licenses of EBQ.
(E-mail: sales@bioneer.com, phone: +82 42 930 8777)

Extendamers™

Description

Extendamers are oligonucleotides from 130 - 200 bases. Bioneer specializes in long oligo synthesis and provides them with Bio-RP or PAGE purification. Extendamers are QC tested by PAGE after oligo synthesis, ensuring the highest quality products for your research needs. Bio-RP (or PAGE) purification also ensures you have only full length oligonucleotides for your experiments. Use Extendamers for cloning, mutagenesis and other demanding applications.

Features and Benefits

- Bio-RP purification or PAGE purification: Only full length oligos
- Use for a variety of applications: Ideal for cloning, gene construction and ddRNAi
- Competitive pricing: Great value for your research dollar

Guaranteed Yield and Delivery Time

Base Limitation (mer)	Guaranteed Yield (nmole)		Delivery Time (Days)	
	BioRP	PAGE	BioRP	PAGE
130 - 200	3-4	0.25-0.3	5 - 6	7 - 8

* The synthesis scales refer to the initial starting point for a synthesis. The starting synthesis scale and the length of the oligo will influence the final yield.

01
DNA
Oligonucleotide
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Large Scale Oligonucleotide

Description

Bioneer's production facilities can accommodate large scale oligonucleotide synthesis orders ranging from milligrams to tens of grams of the purest DNA and RNA oligos, including both standard and modified. A large portion of our bulk oligo customers are involved in research requiring antisense oligos, which has opened the door to new approaches in the development of pharmaceuticals and target validation. Some of the frequently used modifications are:

Phosphorothioates and Chimeric Oligos:
General oligonucleotides are subject to rapid degradation by nucleases. Therefore, oligos for antisense application are usually synthesized with a phosphorothioate bond modification to make them resistant to nuclease activity. In phosphorothioates, a sulfur atom replaces a binding oxygen in the oligo phosphate backbone.

2' O-Methyl RNA oligos:
2' O-Methyl RNA increases nuclease stability and affinity of the antisense oligo to the target RNA.

Features and Benefits

- Free Bio-RP purification: Near HPLC purity at a standard oligo price.
- Broad range of modifications available
- Competitive pricing: Great value for your research dollar

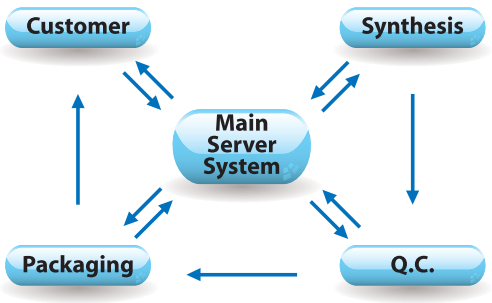
QC and Order System

At Bioneer, quality control is fundamental to our manufacturing processes and guarantees high quality product. Bioneer's Quality Control for Oligonucleotides can be divided into three distinct areas:

■ Bioneer's Olignucleotide Ordering System

Orders from customers are gathered on a main production server system prior to synthesis. To eliminate re-entry errors, on-line and e-mail orders are recommended. Orders are automatically distributed (batched) to an appropriate synthesizer according to the length of oligo, the type of modification, and user's plate choice. Every lot to be synthesized is labeled with its own Barcode ID, which is used for identifying the oligonucleotide plate through the synthesis process. Bioneer's Quality Assurance Staff can monitor all procedures from synthesis to aliquoting using our proprietary Automatic Oligonucleotide Production System (AOP System).

■ The Bioneer QC system



The customer order data is initially saved on the main server system and then transferred to synthesis. Following synthesis, oligonucleotides are spotted on MALDI-TOF mass plates using a proprietary, fully automated, 384-well sample OD quantification/ dispensing robot developed by Bioneer. The MALDI-TOF plates are then transferred to QC Division. Any transfer of oligo samples and plates between different divisions and/or equipment requires the bar-code on each sample racks to be checked by a production specialist to confirm the oligo data. Barcoding ensures compliance and allows related divisions to easily retrieve important oligo data from the main server system.
After receiving the oligonucleotides and all the related information, the QC department checks the quality of the oligonucleotides. The mass spectrum of each oligo is saved and the QC program checks whether the oligonucleotides have been synthesized appropriately. Upon completion the final QC data is transferred to the main server.
The Bioneer QC Program is also used to confirm oligo contaminants (including truncated oligonucleotides) present in the MALDI-TOF spectra. Another key advantage of Bioneer's QC system is its ability to automatically insert mass spectra (0.6X mass - 1.3X mass) of each oligo into the oligo data sheet. Mass spectrum for all ordered oligonucleotides will be provided to customers at no extra charge.
Please note that since the Sequenom Spectrocheck system, which is applicable for some SNP users, employs its own program for oligo QC, $100 USD will be charged for every 96 samples if this QC system is required. Mass spectrum data of examined samples can be provided on a CD if required.

MALDI-TOF data is delivered from QC to the main server, and subsequently all the related information is delivered to packaging so that the correctly synthesized samples will be delivered in the appropriate format as requested by the customer. Bioneer delivers oligonucleotides in a selection of different tubes, 96-well plates, or 384-well plates as per the customers' preferences. After packaged completely, all the oligonucleotides will be shipped by FedEx or UPS, and via their tracking systems, customers may monitor the exact place where the ordered oligonucleotides are in transportation.
In QC, data on all failed samples are automatically returned to synthesis and the re-synthesis of the failed oligonucleotides proceeds whilst QC examines the failed oligo further. This rapid exchange of related information is a key to Bioneer's rapid oligo turnaround time.

Automatic MALDI-TOF QC
Bioneer employs multiple MALDI-TOF mass spectrometers that are fully automated from loading to mass determination. The mass spectrometry data for each sample is automatically inserted into the oligo information sheet. Bioneer is one of the few oligo producers that checks all oligonucleotides (single and high throughput orders) by MALDI-TOF and provides mass data with each oligo, free of charge.

01
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QC and Order System

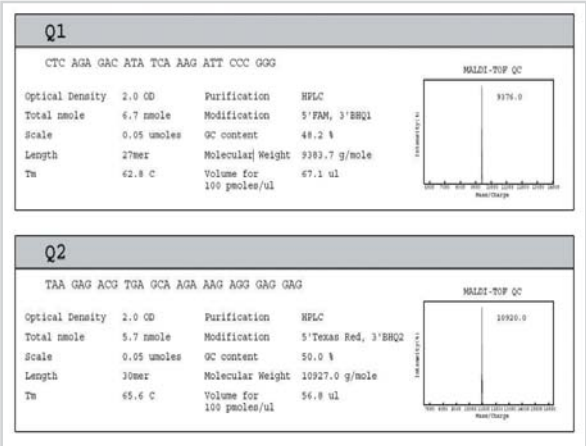

Figure 1. Typical Oligo Datasheet with MALDI-TOF Information

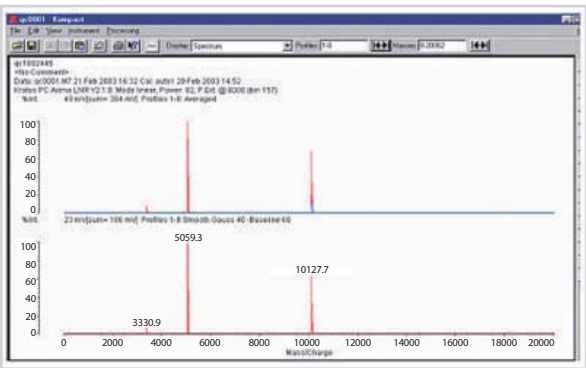

Figure 2. Examples of a typical 30 mer and 23 mer oligo spectrum, in this case employing a Kratos MALDI-TOF system.

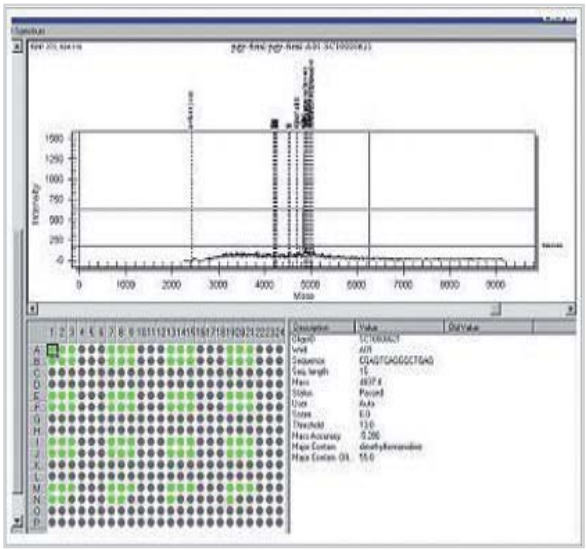

Figure 3. Example of High Throughput Plate QC Analysis. The mass spectrum and result of 92 (plate) oligonucleotides – Bioneer can also provide "spectrochecked" oligo QC data for users of the Sequenom SNP Analysis System.

MALDI-TOF QC System

The Use of Matrix-Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry of Synthetic Oligonucleotide QC

At Bioneer MALDI-TOF (Matrix Assisted Laser Desorption-Ionization Time of Flight) is the technology used extensively for failure detection and other problems that cannot be resolved by other methods. Bioneer's fully automated, high throughput QC systems allow the company to provide superior, high quality product superior to that of our competitors. The QC systems installed at Bioneer currently can check the quality of 35,000 synthetic oligonucleotides per day. Every oligo is supplied with an oligo data sheet that includes MALDI-TOF mass spectrum.

Interpreting MALDI-TOF Mass QC for Oligonucleotide

A MALDI-TOF mass spectrometer accurately measures molecular weight of a sample. This technique is the most useful because it compares the theoretical mass calculated on the basis of oligonucleotide sequence to actual measured data. MALDI-TOF can also be used to check for sequence errors that may occur while inputting sequences. Such a QC method is an absolute requirement for sequence dependent experiments, such as PCR, cloning and sequencing. It can also be used to check whether an oligo has been modified correctly. CE or HPLC analysis cannot be

QC and Order System

used to check modifications. MALDI-TOF is also used to check for the presence of truncated oligonucleotides and salt contamination.

A MALDI-TOF mass system is the most suitable for the QC of oligonucleotides less than 50 bases long.
Longer oligonucleotides (> 50 mer) cannot be ionized effectively (100%) by the laser, therefore they cannot be easily detected and will show a poor detection signal that may fail QC. At Bioneer any oligo longer than 50 bases are checked for quality by PAGE. PAGE QC data sheets are provided with each oligo > 50 bases.

HPLC

HPLC Analysis of Oligo Purity

Reverse Phase HPLC
At Bioneer Reverse Phase HPLC is mostly used to QC of intermediates or single stranded DNA produced in the oligo synthesis process. It is a simple QC technique for modified oligo with hydrophobic groups. Reverse Phase is faster and cheaper than Ion Exchange methods and requires less sample.

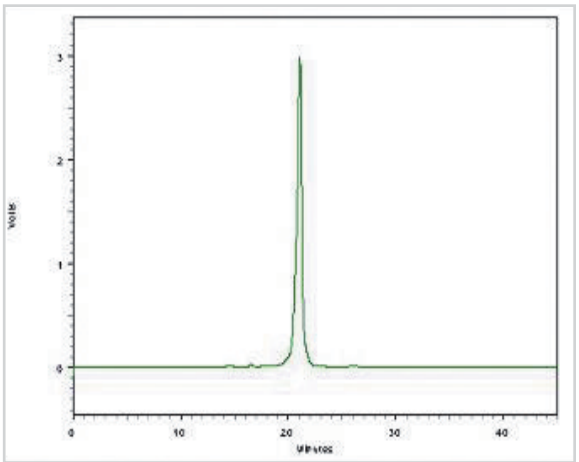

Figure 4. Example of oligo (26 mer) purity analysis using a Reverse Phase (C-18) Column.

Purity Analysis using Ion-exchange Chromatography Method (using Anion-exchange column)
HPLC, equipped with a DIONEX's DNAPac column, is used to QC of oligonucleotides, in particular - Decoy oligonucleotides. The high resolution capability of Ion-exchange can easily separate single stranded DNA and double stranded DNA. At Bioneer Ion-exchange chromatography is commonly used to QC decoy oligonucleotides, and plays a key role in QC confirmation with strict QC standards required for gene therapy.

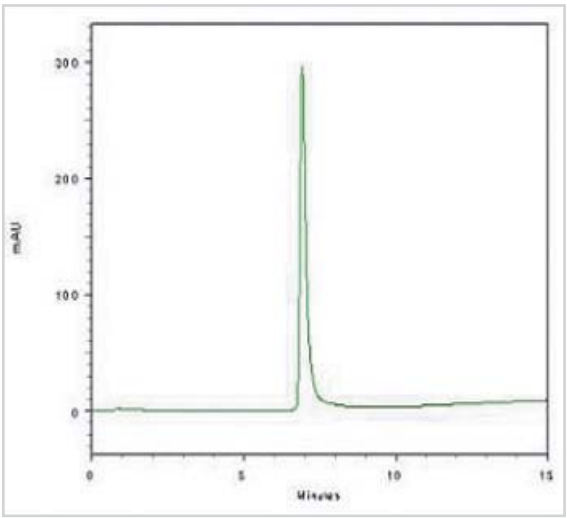

Figure 5. Double stranded DNA (DS DNA) test. Double stranded oligonucleotides are used for decoy or EMSA experiments. Prior to any experiments, the formation of DS DNA must be checked. For decoy experiments used in the development of new drugs, it is necessary to check the ratio of DS DNA in the decoy. In order to guarantee the medical efficacy, the medicine should be formed in decoy like API from the start of drug development. DS DNA confirmation is an FDA requirement.

GC

Product Purity Test Under Gas Chromatograph

Gas Chromatograph (GC) is used to QC for solvent content in Decoy oligonucleotides and S-oligos used in gene therapy. Prior to administering any oligo based drug to humans, it is vitally important to check for the presence of residual organic solvents that may remain after synthesis and purification. Solvent content may compromise efficacy and cause unwanted side effects. The types of residual organic solvents that may be present include acetonitrile, pyridine and toluene etc. Concentrations should be minimally less 0.1%.

01
DNA
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QC and Order System

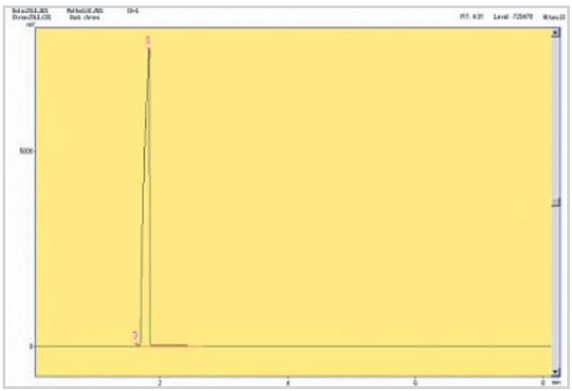


Figure 6. Standard solvent data.

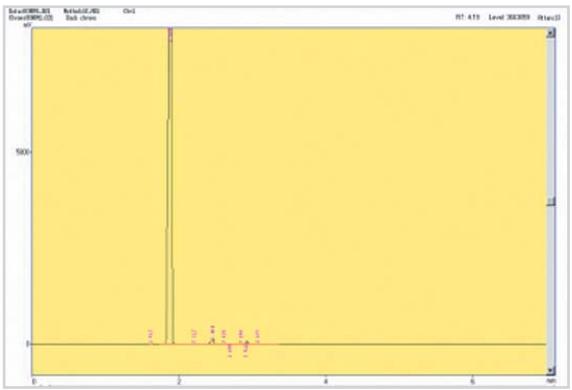


Figure 7. Solvents confirmation data in antisense oligonucleotide

■ QC of Large Scale, Antisense and Decoy Oligonucleotides

Orders from customers are gathered on a main production server system prior to synthesis. To eliminate re-entry errors, on-line and e-mail orders are recommended. Orders are automatically distributed (batched) to an appropriate synthesizer according to the length of oligo, the type of modification, and users' plate choice. Every lot to be synthesized is labeled with its own Barcode ID, which is used for identifying the oligonucleotide plate through the synthesis process. Bioneer's Quality Assurance Staff can monitor all procedures from synthesis to aliquoting using our proprietary Automatic Oligonucleotide Production System (AOP System).

1. Ion-Exchange HPLC analysis
2. Reverse Phase HPLC analysis
3. Capillary Electrophoresis
4. NMR analysis
5. Moisture content analysis
6. Sodium content analysis
7. Heavy metal content analysis
8. Solvent content analysis
9. Endotoxin test
10. Bioburdent test

NMR – Spectroscopy

Nuclear Magnetic Resonance (NMR) spectrometer plays a very important role in understanding 3-dimensional structures of molecules. With increasing interests in the structure of biological materials, the use of NMR spectrometer is expanding into new areas, such as drug development, DNA analysis, human genomic and proteomic research and so on. NMR is commonly used to determine physical structure at the molecular level.

At Bioneer NMR is used for 31P-NMR measurement to compare typical frequency values for phosphates present in DNA backbones. By comparing actual frequencies with theoretical it is possible to check the state and purity of phosphates in synthetic oligonucleotides.

Heavy Metal Testing

For antisense and decoy oligonucleotides that are directly injected into animals or humans as medicines in the pre-clinical or clinical phase, it is necessary to check for heavy metal groups that may influence the efficacy or may cause side effects. The types of heavy metals that require QC may differ in each oligo. Inductively Coupled Plasma-Optical Emission Spectrometers (I.C.P), Atomic Absorbance Spectrophotometers (AAS) and I.C.P Mass Spectrometers are routinely employed to QC oligonucleotides for heavy metal groups. Upon requests, Bioneer's oligonucleotides can be checked quantitatively/qualitatively for metals such as Lead, Nickel and Fe etc.

QC and Order System

Water Content Analysis

Bioneer can also QC oligonucleotides for water content. A Sartorius' Water Content Measurement instrument (MA-30) is employed to measure water contents that may remain in synthesized antisense oligo following the final drying step of the oligo purification process.

Bioburden Testing

Bioneer confirms the sterility of an aseptic oligo production environment by routinely conducting microbial testing of the water used in the synthesis process and final aliquoting steps. Susceptible areas of potential microbial contamination in the synthesis process and the environment, including operators are also checked periodically. Prevention ensures that the final oligonucleotides will be proven to be safe and free from microbial contaminations.

Endotoxin test

Bioneer also utilizes a Kinetic Chromogenic Analysis (KCA) method to confirm that the oligonucleotides are free of any exothermic materials. Generally exothermic materials present in injectable therapeutics are endotoxins from microbial contamination, especially from Gram negative bacterial contamination and must be avoided.

Kinetic Chromogenic Analysis (KCA) is based on an enzyme linked color reaction (limulus Amoebocyte Lysate reaction). The presence of endotoxins is quantified by measuring color of the reaction against known standards. Many samples can be quantified simultaneously using a standard micro-plate reader. KCA is a fast, cost-effective and short measurement. With such a method, Bioneer only provides oligonucleotides with less than 0.25 EU/ml for therapeutic applications.

01 DNA Oligonucleotide (AccuOligo®)

DNA Oligonucleotide FAQs

Handling and Storage

1. How should I store my oligo?

Normally, oligos should be stable at -20°C and can be stored at that temperature for more than a year. Although stable in solution, oligos will be degraded if the storage solution is contaminated with nucleases. Therefore, we recommend that oligos be stored in the dried form. If you want to store oligo in solution, it is best to aliquot the oligo into several tubes and store them separately. Oligos can also be subject to degradation due to the 'Freezing and Thawing Effect' when the oligo solutions are frozen and thawed repeatedly. For storage of DNA, pH value should be maintained at neutrality. Under acidic conditions, DNA can become depurinated. On the other hand, the phosphodiester bond of RNA can be hydrolyzed under basic conditions.

Oligo Shelf life

Storage Condition	Shelf Life (Month)
at RT in water	2
at 4℃ in water	9
at -20℃ in water	18
at -20℃ (Dry)	24

2. How should I resuspend my oligo?

For long-term storage we recommend that the oligos be dissolved in a buffer, such as TE (10 mM Tri-HCl, 0.1 mM EDTA, pH 8.0), instead of just sterilized water. Once resuspended, oligos should be kept frozen at -20°C. Since some oligos may not be easily dissolved in sterilized water, the addition of NaOH does help dissolve oligos in water.

3. If oligos were left at room temperature for more than a week, would they still work?

Once dried, oligos are supposed to have tremendous stability. Even in solution, they are reasonably stable. Therefore, in most cases, without contamination by materials which can cause decomposition of oligos, they should still work well, even if they were left at room temperature for more than a week.

4. Do I have to treat fluorescent dye modified oligos differently in storage and handling?

If exposed to light, fluorescent dye-modified oligos are more fragile than unmodified oligos, and their fluorescence intensities will decrease over time. To maintain their fluorescence efficiencies, fluorescent dye-modified oligos should be stored in the dark at -20°C.

Quantity and Concentration

1. How does Bioneer quantify my oligo?

The quantity of oligo we provide is based on its UV optical density (OD) measured at a wavelength of 260 nm.

2. How do I calculate the oligo quantity from the measured OD value?

After measuring the amount of 260 nm UV light absorbed by the synthesized oligonucleotide, the following formula is used to calculate the actual amount.

$O.D. = \varepsilon C$

ε is the extinction coefficient which is a material-unique constant signifying the amount of light that can be absorbed by a specific material, and C is the concentration of the oligonucleotides. If one knows the extinction coefficient and the O.D. value of the oligonucleotide, the concentration can be calculated by substituting those values in the formula above. The ε value of an oligonucleotide can be calculated in two ways: 1) the sum of extinction coefficients of each base (Table. 1) and 2) an extinction coefficient value calculated by considering sequence interference. The extinction coefficients for each method at 260 nm UV are as below:

Table 1.

dA	15,400
dC	7,400
dG	11,500
dT	8,700

DNA Oligonucleotide FAQs

Table 2.

5' -> 3'	dA	dC	dG	dT
dA	27,400	21,200	25,000	22,800
dC	21,200	14,600	18,000	15,200
dG	25,200	17,600	21,600	20,000
dT	23,400	16,200	19,000	16,800

Therefore, the extinction coefficient may be different, depending on the calculation method.

3. If the O.D. value of 18 mer oligo containing 3dG, 4dC, 5dA and 6T is 0.7, how much oligo is there?

The calculated amount of oligonucleotide depends on the method of extinction coefficient calculation.

Extinction coefficient based on Table 1

e = Number of G bases * 11500 + Number of C bases * 7400 + Number of A bases * 15400+ Number of T bases * 8700
e = 11500 x 3 + 7400 x 4 + 15400 x 5 + 8700 x 6 = 193.3 (ml/mole)
Therefore, substituting the values in the formula O.D. = e ε C leads to
C = 0.7 /193.3 = 0.0036 (mole/ml) = 3.6 (nmole/ml).

Extinction coefficient based on Table 2

e= (εGG+ εGG+ εGC+ εCC+ εCC+ εCC+ εCA+ εAA+ εAA+ εAA+ εAA+ εAT+ εTT+ εTT+ εTT+ εTT+ εTT) - (ε G ε GεC ε C ε C ε A ε A ε A ε A ε T ε T ε T ε T)
e=(21600 + 21600 + 17600 +14600 + 14600 + 14600 + 21200 + 27400 + 27400 + 27400 + 27400 + 22800 + 16800 + 16800 + 16800 + 16800 + 16800) - (11500 + 11500 + 7400 + 7400 + 7400 + 7400 + 15400 + 15400 + 15400 + 15400 + 15400 + 8700 + 8700 + 8700 + 8700 + 8700) = (342,200) -(173,100) = 169,100(ml/mole)
Therefore, substituting the values in the formula O.D. = e ε C leads to
C = 0.7 /169.1 = 0.004139562 (mmole/ml) = 4.14 (nmole/ml).

Our company implements the method of Table 2 for concentration calculation.

4. How do we calculate the molecular weight of oligonucleotide?

The molecular weight of oligo can be calculated with the following equation:
M.W. = NA * 249.2 + NC * 225.2 + NG * 265.2 + NT * 240.2 + (oligo length-1) * 63.98 + 2.02
NA = Total # of A; NC = Total # of C; NG = Total # of G; NT = Total # of T

5. How do I convert oligo quantity expressed in nmole into weight?

Normally, the amount of synthetic oligonucleotide is described in number of moles, usually nmole. The amount of oligo can easily be calculated from the following equation: Amount of oligo (ng) = Molecular Weight (M.W. in g) X Number of moles (nmole)

6. If I do not know the exact base composition, is there any method to quantify the synthesized oligo?

Approximately - a single stranded oligo with 1 O.D. value contains 33 μg while double stranded oligo contains 50 μg. For short oligos, however, there would be big deviations from the above values.

7. How do I measure Tm of the synthesized oligo?

Tm (melting temperature) refers to the temperature where 50% of oligonucleotides exist in duplex form and the rest in single-strand form.
There are several ways to calculate Tm. At Bioneer, we use the nearest-neighbor method (PNAS 83, 3746-50).
It is believed that the effect of hybridization is different for every sequence and that through thermodynamic measurements; you can estimate Tm values more accurately.
For example, the sequences of 5'-GC-3' and 5'-CG-3' are different in thermodynamic measurements.

The method for nearest-neighbor calculation is as follows:
Through thermodynamic measurements, enthalpy and entropy values are determined between 2 bases. [Salt] is the concentration of monovalent cations and [Oligo] is the oligo concentration. R is the gas constant (1.987 cal•K-1mole-1).

01 DNA Oligonucleotide (AccuOligo®)

DNA Oligonucleotide FAQs

Bioneer's Tm value is calculated by the nearest-neighbor method with 50 mM for salt concentration and 1 nM for oligo concentration.
Please note that there are other ways of estimating the Tm. For oligos shorter than 15 mer, the Wallace rule can be used:

Tm = 2℃ (A + T) + 4℃ (G + C)

Another estimation method based on the GC content for long sequences is:
Tm = 81.5 + 0.41(%GC) - 500/L + 16.6 log[M]
(L; oligonucleotide length, [M]; monovalent cation concentration)

However, these methods do not consider the base stacking effect and usually the estimation is not as accurate as the nearest neighbor method. Nonetheless, there are still some disadvantages in the nearest neighbor method for 60-70 or under 15 bp estimation.
Bioneer provides the Tm values of every oligo, but the values are estimations and we cannot guarantee the exact values. Therefore, the Tm value should be used only as a reference.
If the experiment does not yield anticipated results, it is recommended to lower the annealing temperature by 4-5 degrees from the Tm value. If there are many non-specific products, trial-and-error approach should be taken to obtain the optimum annealing temperature.

8. Why are there differences in Tm value that Bioneer provided and mine?

The TM Calculator that Bioneer uses is different from, and more accurate than, the more commonly used calculators based on the Wallace rule.

9. What is the method for adjusting the oligonucleotide concentration?

On the data sheet that Bioneer provides for each oligo, the volume of TE buffer or distilled water necessary to make a 100 pmole/μl oligo solution appears by the "volume for 100 pmole/μl" heading.

For example, if 189.0 is indicated on the sheet, add 189μl of TE buffer to the tube.
Such a solution would be 100 μM in concentration:
100 pmole/μl = 100 x 10^{-12} mole/10^{-6}ℓ
= 100 x 10^{-6} mole/ℓ
= 100 x 10^{-6} M
= 100 μM

10. Unit conversions

System of scientific units:

10^{-1} = deci [d]
10^{1} = deca [da]
10^{-2} = centi [c]
10^{2} = hecto [h]
10^{-3} = milli [m]
10^{3} = kilo [k]
10^{-6} = micro [u]
10^{6} = mega [M]
10^{-9} = nano [n]
10^{9} = giga [G]
10^{-12} = pico [p]
10^{12} = tera [T]
10^{-15} = femto [f]
10^{15} = peta [P]
10^{-18} = atto [a]
10^{18} = exa [E]
10^{-21} = zepto [z]
10^{21} = zetta [Z]
10^{-24} = yocto [y]
10^{24} = yotta [Y]

Example for unit exchange of oligonucleotides
1 pmole/μl
= 1x10-12 mole / 1x10^{-6}ℓ
= 1x10^{-6} mole / ℓ
= 1 μmole / ℓ
= 1 μM

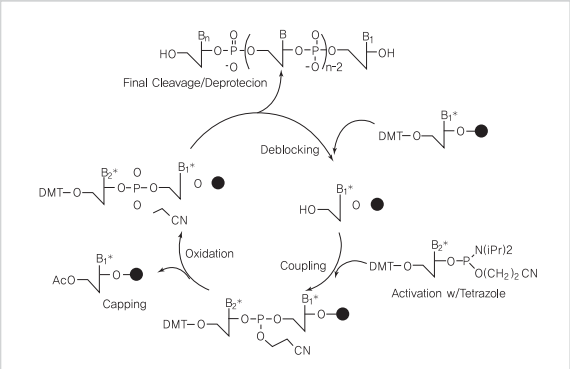
DNA Oligonucleotide FAQs

Synthesis and Order

1. How are oligonucleotides synthesized?

The most popular method for synthesizing oligonu - cleotides is to form natural 3'-5' phosphodiester bonds between monomers by using 'phosphite triester' protocols. ß-cyanoethyl phosphoramidites, the building monomers, were developed by Koster and used most often to synthesize oligonucleotides (Nucl. Acids Res. 1984, 12, 4539; Tetrahedron Lett. 1983, 24, 5843). Through the 'phosphite triester' method using ß-cyanoethyl phosphoramidite, high coupling efficiency is achieved (> 98%) and the time consumed for coupling is much shorter than that of other methods of oligo synthesis. Moreover, since the monomer ß-cyanoethyl phosphoramidite is quite stable prior to the activation, which is necessary for oligo synthesis, and means they can be stored for a long period of time.

The oligonucleotide is synthesized while attached covalently to a solid support. Excess soluble protected nucleoside β-cyanoethyl phosphoramidites and coupling reagent can drive the reaction near to completion. Among the solid supports, controlled pore glass (CPG), which consists of a glass matrix prepared uniformly with pores of defined size, has been used predominantly over the last few years.



<Figure 1 >

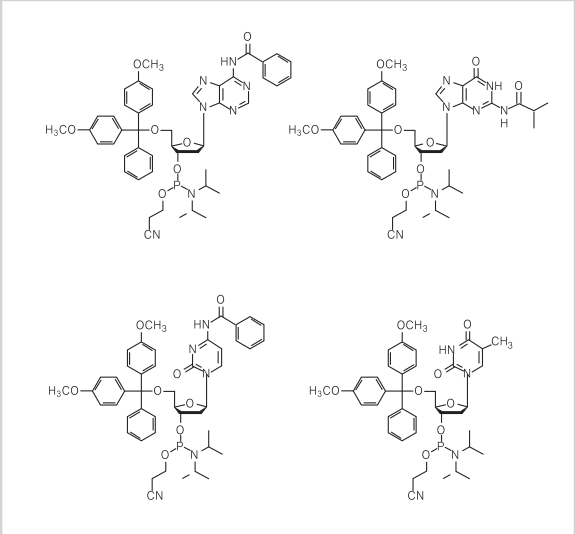
The whole synthesis of oligonucleotides can be accomplished by the chain reactions where four different reaction cycles - deblocking, coupling, oxidation and capping are performed repeatedly (Figure 1).

Deblocking

In the first step in of synthesis - deblocking - the 5' protecting group, DMT, is cleaved from the CPG. For deblocking, acidic condition is necessary, and trichloroacetic acid (3 % in dichloromethane) is used in most of cases. It is reported that oligos can be depurinated in acidic conditions, especially more severe for adenosine. Since trichloroacetic acid is very acidic (pKa: ~1.5), deblocking solution with trichloroacetic acid should not be left too long in the reaction. Instead of trichloroacetic acid, dichloroacetic acid, which is less acidic than trichloroacetic acid, can be used for deblocking to avoid the depurination problem in certain cases. Since the DMT cation, which is produced after deblocking cycle, shows a very strong orange color, it can be used to monitor the coupling efficiency by measuring its light absorbance.

Coupling

The 5'-hydroxyl group on the CPG, which is exposed after the deblocking step, is coupled to the nucleoside β-cyanoethyl phosphoramidites to form triphosphite ester which is subsequently oxidized to a phosphotriester bond. For nucleoside β-cyanoethyl phosphoramidites, to avoid the unwanted side reaction during the whole oligo synthesis, exocyclic amino groups in base moiety are



<Figure 2 >

01 DNA Oligonucleotide (AccuOligo®)

DNA Oligonucleotide FAQs

protected to result amide structure. Benzoyl groups are used for both adenosine and cytidine. On the other hand, isobutyryl group is used for guanosine base protection. Since thymidine doesn't have exocyclic amine group in base there is no need for extra protection. 5'-Hydroxyl groups are protected with DMT for all nucleoside β cyanoethyl phosphoramidites (Figure 2).

Since nucleoside β-cyanoethyl phosphoramidites are quite stable under normal conditions, they cannot react directly with a free 5′ hydroxyl function on a growing chain. They must first be activated by treatment with an activator usually a type of weak acid. Among a variety of candidates, tetrazole has shown a great efficiency and has been used as a standard activator. Tetrazole has been thought to play a dual role: it protonates the diisopropylamino group of the phosphoramidite function; and then comes in as a nucleophile, generating a very reactive tetrazolophosphane intermediate. Coupling reactions with these activated nucleoside phosphoramidite reagents are very fast (less than 2 minutes) and are almost quantitative.

Oxidation

The newly formed phosphite internucleotide linkage is unstable and susceptible to both acidic and basic cleavage. Therefore, the trivalent phosphite triester is oxidized to a stable pentavalent phosphate triester. Iodine is used as a mild oxidant in basic tetrahydrofuran solution with water as the oxygen donor. The reaction is extremely fast, being quantitative in 30 seconds.

Capping

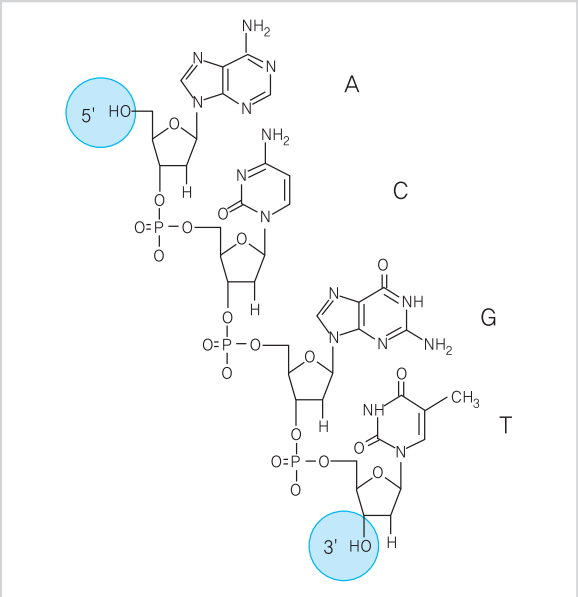
Since the coupling reaction cannot be quantitative in a finite time period, a small percentage of truncated sequences are produced at every coupling step. If these failure sequences were allowed to react further, it would be difficult to isolate the product from the sequence mixture. This problem is overcome largely by capping the remaining free hydroxyls through acetylation.

Acetylation is achieved with the strong acetylation reagent which forms on reaction of equimolar amounts of acetic anhydride and N-methylimidazole. The reaction is almost quantitative in 30 seconds.

After oxidation, the nucleotide addition cycle is complete. Oligonucleotide synthesis can continue removing the DMT group at the 5′ -end of the growing chain and repeating another cycle of nucleotide addition.

At the end of whole synthesis of oligonucleotides, cleavage from support and simultaneous base and phosphate deprotection are achieved by treatment with concentrated ammonium hydroxide.

2. Standard oligo structure.



3. What are base limitations on each synthesis scale?

0.025 μmole synthesis scale: 15 – 60 mer
0.05 μmole synthesis scale: 10 – 75 mer
0.2 μmole synthesis scale: 5 – 110 mer
1.0 μmole synthesis scale: 5 – 130 mer
10 μmole synthesis scale: 5 – 50 mer
15 μmole synthesis scale: 5 – 50 mer

4. I ordered the 50 nmole scale, but I got less than 50nmoles. What happened?

50 nmole scale synthesis of oligos doesn't mean we can guarantee 50 nmole of final oligos. Instead, 50 nmole scale refers to the loading amount of solid support used at the beginning of oligonucleotide synthesis. Since oligos are

DNA Oligonucleotide FAQs

usually ordered by the reaction scale not the final yield, the amounts of oligos which customers could get is naturally less than ordered. The final yields can vary with oligo length, base composition and coupling efficiency.

5. Can you make the oligos having a high percentage of G residues?

It is known that oligos having a high percentage of "G" residues are difficult to synthesize, especially if sequence contains several "G" in a row. It is also reported if there are "G"s existed four or more in a row, oligos tend to aggregate and form "guanine tetraplex". (Poon and MacGregor, Biopolymers, 1998, 45, 427-434) By substitution of inosine for some of "G", the formation of "guanine tetraplex" can be disrupted.

6. Do you provide oligoribonucleotide (RNA) synthesis?

Yes, we do. We can offer oligoribonucleotide with 2′ -OH and/or 2′ -O-methyl structure at the desired site. We can also synthesize the chimeric oligos which have DNA and RNA structures mixed.

7. Does the oligo synthesized have phosphate group at 5′ or 3′ position?

If not ordered separately, the oligos synthesized do not contain phosphate group at 5′ or 3′ position. If you want to have oligo phosphorylated at 5′ or 3′, you should specify 5′ or 3′ phosphorylation modification when ordering.

8. What are the symbols denoting degenerate bases?

R : A or G
Y : C or T
M : A or C
K : G or T
S : G or C
W : A or T
V : A, C, or G
H : A, T, or C
B : G, T, or C
D : G, A, or T
N : A, C, G, or T

01
DNA
Oligonucleotide
(AccuOligo®)

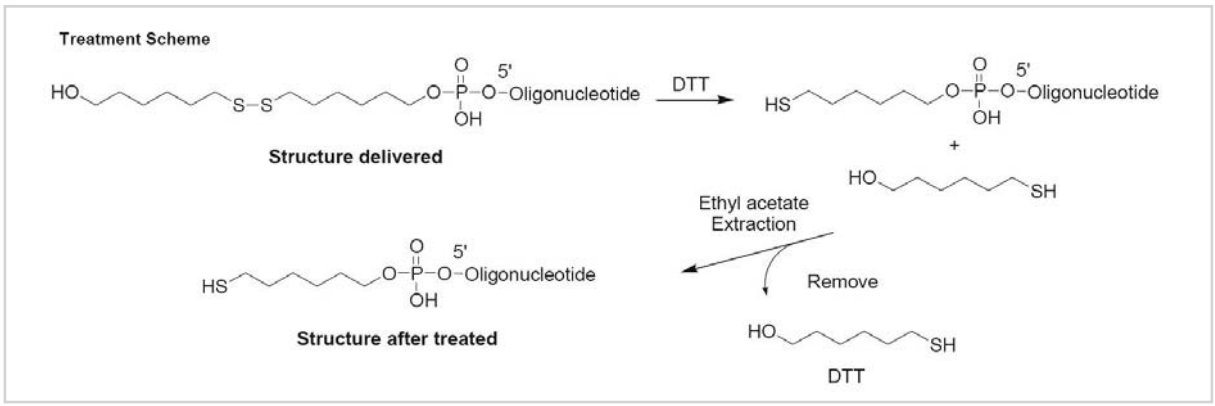
User Protocol

Treat thiol modified oligonucleotides

1. Dissolve the dried thiol-modified oligonucleotide (5 OD based) in distilled water or an appropriate buffer, e.g 0.1M TEAA pH 7.5 (50 µl)

2. Add 10 µl of 1.0 N dithiothreitol (DTT), vortex, then incubate at room temperature for 15 minutes.
(1.0 N DTT: 0.01 M Sodium acetate (pH 5.2) 20 ml + 1.545g DTT dissolve (filtration))

3. Remove excess DTT and unwanted thiol fragments from the thiol-modified oligonucleotide mixture by extracting with ethyl acetate 3 times, using 50 µl per extraction. Discard the upper layer after vortexing the mixture. You must immediately proceed to the next step since the free sulfhydryl group becomes unstable after the removal of DTT.



User Protocol

Anneal complementary pairs of oligonucleotides

General Procedure

1. Mix the concentrated complementary oligonucleotides together at 1:1 molar ratio in a micro centrifuge tube.

2. Dilute the oligonucleotide mixture to a final concentration of 1 pmol/μl with Tris or phosphate buffer containing salts.

e.g.10 mm Tris, 0.1 mm EDTA, 50 mm NaCl (pH 8.0) or 100 mm sodium phosphate, 150 mm NaCl, 0.1 mm EDTA (pH 7.5 or 8.0).

3. Anneal the oligonucleotides using one of the annealing methods described below.

4. Aliquot and store at -20°C. The double-stranded DNA probes may be stored at 4°C for several weeks, given that care is taken to protect the probes from nuclease degradation.

Annealing Methods

• Option 1: Anneal with a heating block

1. Incubate the oligonucleotides at 95°C for 5 minutes.

2. Gradually reduce the heat until the oligonucleotides have reached room temperature.

• Option 2: Anneal with a water bath

1. Boil 400 ml of water in a large glass beaker on a hotplate.

2. Incubate the tube of oligonucleotides in the boiling water for 5 minutes.

3. Turn off the hotplate, leaving the oligonucleotides in the beaker on the hotplate to slowly cool to room temperature.

• Option 3: Anneal with a thermal cycler

1. A thermal cycler allows for convenient and reproducible annealing of oligonucleotides.

2. Use Table1 as a guide to program your thermal cycler for either a simple or advanced protocol.

3. The notation -1°C/cycle indicates a 1°C decrease in temperature per cycle.

4. Refer to your thermal cycler Operation Manual or consult the manufacturer for information about programming your particular instrument.

		Cycles	Temperature	Time
Simple protocol	Step 1	1	95°C	5 min
	Step 2	70	95°C (-1°C/cycle)	1 min
	Step 3		4°C	HOLD
		Cycles	Temperature	Time
Advanced Protocol (example in which the oligonucleotide pair has a Tm of 55°C)	Step 1	1	95°C	5 min
	Step 2	40*	95°C (-1°C/cycle)	1 min
	Step 3	1	55°C	30 min
	Step 4	20*	55°C (-1°C/cycle)	1 min
	Step 5		4°C	HOLD

* The number of cycles in step 2 and 4 depends on the Tm of the oligonucleotides to be annealed.

Table1. Thermo cycler programs for annealing complementary oligonucleotides.

02 RNA Oligonucleotide(siRNA)

RNA Oligonucleotide (siRNA)

RNA Oligonucleotide (siRNA)

Overview

Recently, gene knockdown or knockout technologies, such as antisense, ribozyme, and gene knockouts were used to perform loss-of-function studies. However, the post-genomics era calls for high-throughput gene function studies which the former technologies were unable to answer due to poor reproducibility, high cost, and excessive time to the result. The advent of siRNA technology has opened up many new possibilities in the field of gene suppression. siRNA has the following advantages over other RNAi technology:

- Reduced time and costs: Less screening is required to obtain highly effective siRNA.
- High efficacy at lower concentration: Lower concentrations provide effective gene silencing and minimizes off-target effects.
- Specificity: siRNA is a highly specific target knockout mechanism based on the natural biological mechanisms of RNAi.

siRNA mechanism

siRNA is the term for 20 - 25-base pair RNA duplexes, where the two terminal 3'-nucleotides are unpaired (3'-overhang). When siRNAs are introduced into cells they combine with a protein complex called the RNA-induced silencing complex (RISC) and are unwound by a helicase. The RISC complex containing single-stranded RNA complementary to the target mRNA then recognizes and binds to the target mRNA. After binding the mRNA, the argonaute protein Ago2 cleaves it and complete degradation of the target mRNA is carried out by ribonuclease activity (as a result of the lack of protection by 5' caps or poly (A) tails). This exciting technology is one of the most effective methods for the silencing of specific target genes and is a must for gene function validation studies, drug target validation, and for gene therapy studies.

02 RNA Oligonucleotide (siRNA)

Custom siRNA

Description

Bioneer's Custom siRNA synthesis service offers exceptional quality siRNAs to knock down your target genes. Custom siRNAs can be synthesized according to sequence information you provide, or you can take advantage of our complimentary siRNA design service. Up to 30-mer siRNA including a choice of 32 different 3' overhangs can be ordered with a variety of modification options for expanded specificity. Bioneer's siRNA is provided purified, annealed, lyophilized and ready-to-use. For even greater convenience, check out our *AccuTarget*™ Genome-wide Pre-designed siRNA - siRNAs pre-designed for human (18,048 genes), mouse (17,118 genes) and rat (9,392 genes) synthesized and ready-to-ship.

All custom siRNAs are synthesized in our state-of-the-art clean room facility and then purified free of charge utilizing Bioneer's BioRP purification system. For higher purity, HPLC purification is available at an additional charge. Each siRNA is quality controlled by MALDI-TOF mass spectrometry to guarantee highest quality (Figure 1) and analyzed by PAGE to confirm its duplex structure (Figure 2).

Features and Benefits

- Guaranteed performance: Two of three custom siRNA will give 80% siRNA knockdown
- Guaranteed Quality: Manufactured in a state-of-the-art clean room and QC'ed by MALDI-TOF and PAGE
- Custom siRNA design service available: Turbo si-Designer software design is available free of charge
- Competitive pricing: Overhang and annealing service provided free of charge, great value for your research dollar

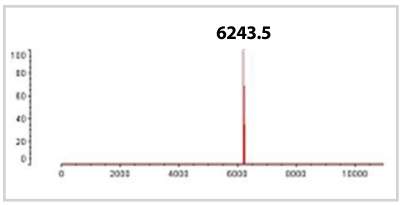


Figure 1. MALDI-TOF mass spectrometry analysis of the synthesized siRNA. All shipped siRNAs are processed through rigorous quality control (QC) procedures, including MALDI-TOF and gel anlysis.

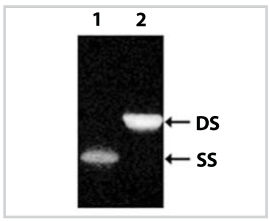


Figure 2. Complementary single-strand RNA strands were annealed to form double-stranded siRNA.
The resulting siRNA was analyzed by 15% non-denaturing PAGE. SS: single-strand RNA, DS: double-strand siRNA.

Ordering Information

Cat. No.	Product Name	Guaranteed Yield	Purification
S-1017-5	Custom siRNA	1 nmole	BioRP
S-1017-6		5 nmole	
S-1017-1		10 nmole	
S-1017-2		20 nmole	
S-1017-3		50 nmole	
S-1017-4		100 nmole	
S-1018-5		1 nmole	HPLC
S-1018-6		5 nmole	
S-1018-1		10 nmole	
S-1018-2		20 nmole	
S-1018-3		50 nmole	
S-1018-4		100 nmole	

Modification

Modification	
5' Fluorescein	3' Amine
5' Phosphorylation	3' TAMRA
5' Biotin	3' Thiol
5' Amine	3' DABCYL
5' TAMRA	3' Cholesterol
5' Thiol	3' PEG 2000
5' PEG 2000	Phosphorothioate
3' Fluorescein	Internal 2'-OMe
3' Phosphorylation	Internal Inosine
3' Biotin	Internal Deoxy-abase

Genome-wide siRNA

Overview

Turbo si-designer: Bioneer's proprietary siRNA design algorithm

Small interfering RNA (siRNA) has recently emerged as a novel tool in the functional genomics area of small RNA molecules (siRNA and microRNA). RNA interference (RNAi) is a mechanism of gene silencing at the mRNA level. This phenomenon is triggered by small interfering (si)RNAs and micro (mi)RNAs. These molecules involved in gene regulation belong to an expanding class of small non-coding RNAs. siRNA is capable of inhibiting gene expression by either directing the degradation of homologous mRNA targets or inducing the repression of translation of mRNA targets.

In 2002, siRNA was hailed by Science magazine as being the "Breakthrough of the year" technology. In RNAi experiments, the most critical design factor is specific target recognition which is critical because the efficiency level of siRNA is different for each site. Silencing the correct gene enables researchers to obtain reproducible experimental results which can lead to the subsequent use of siRNA as a genetic drug. Experimental success depends upon several factors. The most critical among these factors is the design of effective and specific siRNA. Bioneer, incollaboration with the world renowned National Genome Information Center (NGIC) at KRIBB institute, has developed a proprietary siRNA selection algorithm. Turbo si-Designer identifies highly effective siRNA target sites with exceptional success rates. Several important parameters including base composition, the number of repetitive bases in a row, thermodynamic instability, energy profiling and base preference were considered in the development of Turbo si-Designer. The siRNAs spanning SNP sites are removed and non-specific siRNAs are eliminated after BLAST to minimize off-target effects. The resulting candidates are then ranked according to the NGIC scoring system. The performance of the algorithm was evaluated by designing hundreds of siRNAs and testing the siRNA knockdown efficacy by Real-time PCR analysis. Over 80% of the siRNAs tested showed > 75% knockdown of the target mRNA and more than 40% of siRNAs induced > 90% knockdown. Notably, siRNAs with the low NGIC score were mostly nonfunctional, indicating that ineffective siRNAs are efficiently removed by Turbo si-Designer.

To validate the performance efficacy of Turbo si-Designer, Bioneer tested the knockdown efficiency of 82 predesigned siRNAs in anti-apoptosis and cell division related genes (Survivin). The siRNA was transfected into A549 lung carcinoma cells and the knockdown efficiency was then analyzed using QuantiGene ViewRNA Analysis. As seen on the Figure 3, the lower-scoring siRNAs are not effective compared to the higher-scoring siRNA (Figure 3A and 3B.) and thus Turbo si-Designer can predict the higher efficiency siRNA by the exclusion of ineffective siRNA sites.

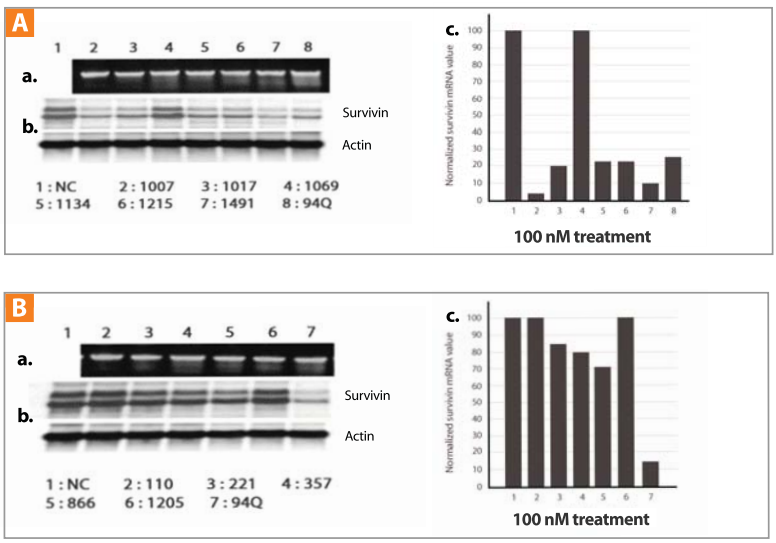


Figure 3. Knockdown efficiency of siRNAs designed by Turbo si-Designer was analyzed by Northern blot and Real-Time PCR analysis. A) Knockdown efficiency of high score siRNAs. B) Knockdown efficiency of low score siRNAs.

02 RNA Oligonucleotide (siRNA)

AccuTarget™ Genome-wide Predesigned siRNA Library

Description

Bioneer offers over 132,000 predesigned siRNAs for more than 44,000 target genes from Human, Mouse and Rat. Search our extensive siRNA library by Gene ID, Symbol, Synonyms, Description, or Accession Number. Once you find your gene of interest, choose your guaranteed yield and purification level, and check your predicted siRNA knockdown efficiency. You can also even order your qPCR primers for siRNA knockdown validation. Convenient and easy siRNA ordering - only from Bioneer.

When purchasing 3 siRNAs for the same gene, Bioneer guarantees at least 80% reduction in the target mRNA level for two of the siRNAs. If there is not a > 80% reduction in the mRNA level of the target gene, Bioneer will provide a replacement of 2 siRNAs free of charge. Bioneer reserves the right to request supporting data inclusive of:

1. siRNA Knockdown efficiency data: NC (*AccuTarget*™ Negative Control) and siRNA concentration at 100 nM, and
2. Transfection efficiency data: PC (*AccuTarget*™ GAPDH/GFP/Luciferase siRNA) and NC (*AccuTarget*™ Fluorescein-labeled Negative Control).

Features and Benefits

- High siRNA knockdown rates: 2 of three siRNAs suppress target mRNA levels > 80%
- Unique design algorithm: Maximizes siRNA knockdown while minimizing off target effects
- Competitive pricing: Great value for your research dollar

Applications

AccuTarget™ Genome-wide Predesigned siRNA library can be used in a variety of RNAi experiments.

- Functional genomics and proteomics research
- Gene expression studies
- Array analysis

Ordering Information

Cat. No.	Product Name	Guaranteed Yield	Purification
SDO-1005	*AccuTarget*™ Genone-wide Predesigned siRNA Library	1 nmole	BioRP
SDO-1006		5 nmole	BioRP
SDO-1001		10 nmole	BioRP
SDO-1002		20 nmole	BioRP
SDO-1003		50 nmole	BioRP
SDO-1004		100 nmole	BioRP
SDH-1005		1 nmole	HPLC
SDH-1006		5 nmole	HPLC
SDH-1001		10 nmole	HPLC
SDH-1002		20 nmole	HPLC
SDH-1003		50 nmole	HPLC
SDH-1004		100 nmole	HPLC

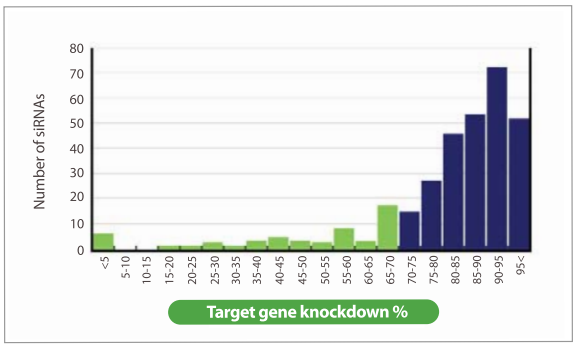


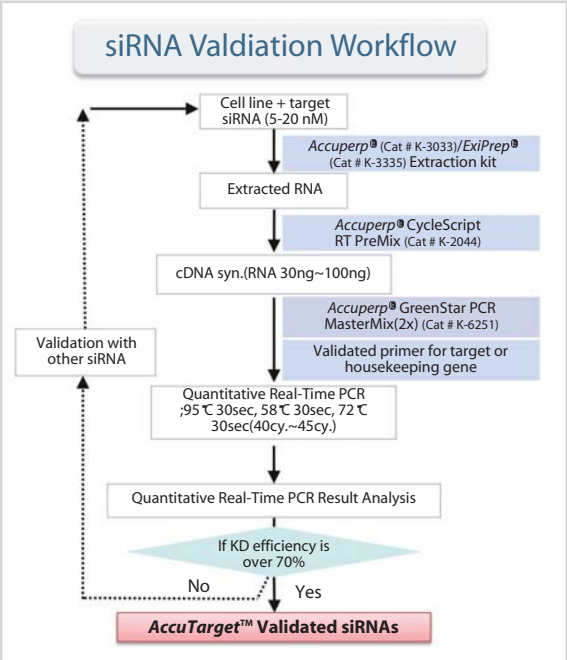
Figure 4. Knockdown efficiency of *AccuTarget*™ Genome-wide Predesigned siRNA

AccuTarget™ Pre-designed siRNAs are Highly Effective, to determine knockdown efficiency of predesigned siRNAs, HeLa cells were transfected with siRNAs at 100 nm concentration. Twenty-four hours post-transfection, total RNA was isolated and the level of target mRNA was measured by qRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm: 83.8% of tested siRNAs induced >70% knockdown and 38.1% of tested siRNAs elicited >90% knockdown.

AccuTarget™ Premade siRNA Sets

■ Description

Bioneer offers the *AccuTarget*™ Premade siRNA Sets, which contain 25,368 predesigned and manufactured siRNAs for immediate use in your experiments. These Premade human siRNA Sets are available at 10, 20, 50 and 100 nmole guaranteed yield. We also offer 25 Pathway-specific / gene family siRNA Sets for researchers studying cellular processes, cancer, and disease etc. These are available at 0.1, 0.25, 0.5 and 1 nmole guaranteed yield. Finally there are 21 pre-validated siRNA libraries with high knockdown rates and demonstrated effectiveness. Validated siRNAs can be ordered at 10, 20, 50 and 100 nmole guaranteed yield.
These designed siRNA validation process is as follows:



■ Features and Benefits

- Categorized by Pathway / family: Convenient format for research
- Pre-validated siRNA libraries available: Works the first time and every time.
- Competitive pricing: Great value for your research dollar

■ Applications

AccuTarget™ Premade siRNA Sets can take advantage of the various RNAi experiments

- Pathway analysis and target identification and validation
- Drug target HTS (High-Throughput siRNA Screening)

02 RNA Oligonucleotide (siRNA)

AccuTarget™ Premade siRNA Sets

AccuTarget™ Premade Human siRNA Sets

Cat. No.	Product Name	No. of Genes	Cat. No.	Product Name	No. of Genes
SHS-001	Antioxidant siRNA Set	38	SHS-013	Lyase siRNA Set	123
SHS-002	Apoptosis siRNA Set	290	SHS-014	Motor siRNA Set	122
SHS-025	Cancer siRNA Set	1157	SHS-015	NF-kB pathway siRNA Set	37
SHS-003	Caspase siRNA Set	37	SHS-016	Nucleic acid binding siRNA Set	2573
SHS-004	Cell cycle siRNA Set	112	SHS-017	Oxidoreductase siRNA Set	551
SHS-005	Cyclase siRNA Set	22	SHS-018	Peptidase siRNA Set	491
SHS-006	Cytochrome P450 siRNA Set	52	SHS-019	Phosphatase siRNA Set	188
SHS-007	Deaminase siRNA Set	22	SHS-020	Receptor siRNA Set	1516
SHS-008	GPCR signaling pathway siRNA Set	727	SHS-021	Transferase siRNA Set	1428
SHS-009	Helicase siRNA Set	114	SHS-022	Transporter siRNA Set	1021
SHS-010	Isomerase siRNA Set	104	SHS-023	Tubulin siRNA Set	20
SHS-011	Kinase siRNA Set	700	SHS-024	Ubiquitin siRNA Set	77
SHS-012	Ligase siRNA Set	272			

The results of the evaluation indicated the Bioneer's design algorithm was highly effective in selecting effective siRNAs; 80% of the tested siRNAs showed > 70% knockdown and 38% elicited knockdown of > 90%. (Figure 5).

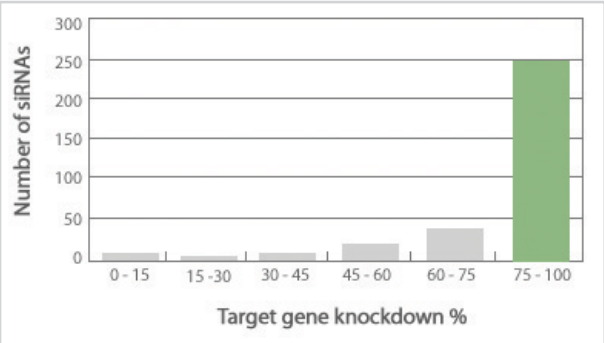


Figure 5. Knockdown efficiency of *AccuTarget*™ Premade siRNA Sets.

AccuTarget™ Premade siRNA Sets are highly effective to determine knockdown efficiency of library siRNAs, HeLa cells were transfected with siRNAs at 100 nm concentration. Twenty-four hours post-transfection, total RNA was isolated and the level of target mRNA was measured by qRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm: 83.8% of tested siRNAs induced >70 % knockdown and 38.1% of tested siRNAs elicited >90 % knockdown.

AccuTarget™ Validated Human siRNA Sets

Cat. No.	Product Name	No. of Genes	Cat. No.	Product Name	No. of Genes
SHV-002	Apoptosis Validated siRNA Set	208	SHV-015	NF-kB pathway Validated siRNA Set	34
SHV-025	Cancer Validated siRNA Set	466	SHV-016	Nucleic acid binding Validated siRNA Set	144
SHV-003	Caspase Validated siRNA Set	18	SHV-018	Peptidase Validated siRNA Set	27
SHV-004	Cell cycle Validated siRNA Set	107	SHV-019	Phosphatase Validated siRNA Set	119
SHV-009	Helicase Validated siRNA Set	8	SHV-020	Receptor Validated siRNA Set	53
SHV-011	Kinase Validated siRNA Set	104	SHV-021	Transferase Validated siRNA Set	117
SHV-012	Ligase Validated siRNA Set	27	SHV-022	Transporter Validated siRNA Set	17

AccuTarget™ Premade siRNA Sets

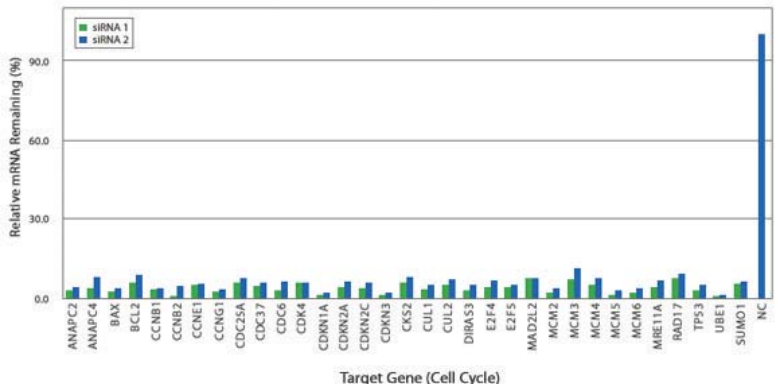


Figure 6. Efficient knockdown by *AccuTarget*™ Validated siRNAs (20 nm siRNA concentrations for Cell cycle Validated Set)
AccuTarget™ Validated Human Cell cycle siRNA Set is highly effective to determine knockdown efficiency of library siRNAs, HeLa cells were transfected with siRNAs at 20 nm concentration. Twenty four hours post-transfection, total RNA was isolated and the level of target mRNA was measured by QRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm.

02 RNA Oligonucleotide (siRNA)

AccuTarget™ Control siRNAs

■ Description

AccuTarget™ Positive Control siRNAs are designed to induce high siRNA knockdown of their target genes (Figure 6, 7 & 8). siRNAs targeting an endogenous gene (GAPDH) or a reporter system (GFP and Luciferase) are available. *AccuTarget*™ Negative Control siRNAs do not target any known genes in human, mouse and rat. The negative control siRNA can be fluorescently labeled for easier monitor of transfection efficiency. *AccuTarget*™ Control siRNA Sets consisting of a positive and negative control siRNAs are also available for user convenience (Figure 9).

■ Features and Benefits

- Excellent performance: Positive control siRNA knockdown rates > 90%
- Monitoring of transfection rate: Convenient fluorescent-labeled negative control Sets
- Competitive pricing: Great value for your research dollar

Positive control

1. GAPDH-siRNA

A) Northern blotting

B) qRT-PCR

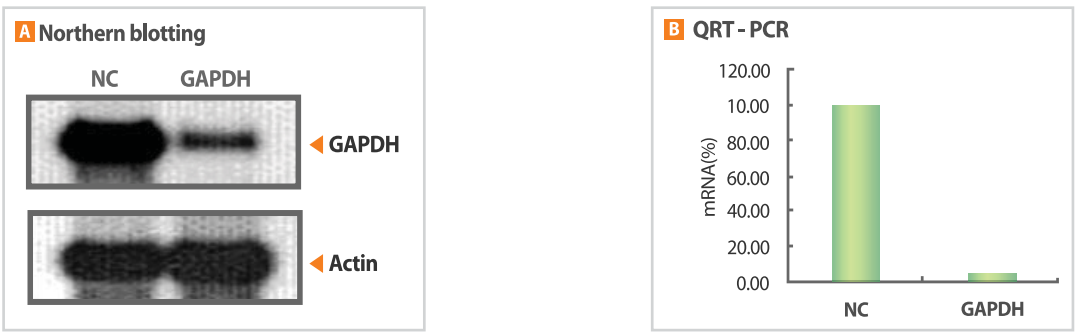


Figure 7. HeLa cells were transfected with GAPDH- and NC(negative control)-siRNA at 100 nm final concentration using lipofectamine 2000 reagent. Twenty-four hr post-transfection, total cellular RNA was isolated from transfected cells and subjected to Northern blot and Real-Time PCR analyses. As can be seen in Fig. 7B, only about 3% GAPDH mRNA remained after 24 hr treatment with GAPDH siRNA, indicating that highly efficient knockdown of GAPDH mRNA can be easily achieved using our positive control GAPDH siRNA.

2. GFP-siRNA

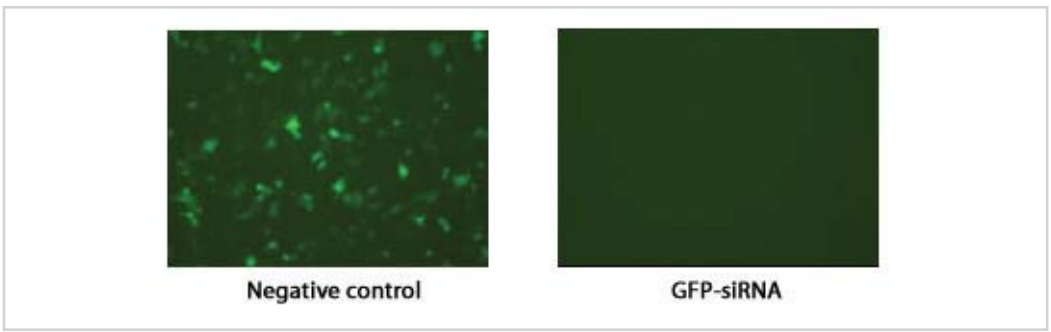


Figure 8. HeLa cells in a 24-well plate were cotransfected with 200 ng of CMV-GFP plasmid and 10 nm of GFP siRNA using lipofectamine 2000 transfection reagent. Next day, the expression of GFP was observed by using a Nikon Eclipse TS100 epifluorescence microscope. In contrast to bright green fluorescence of GFP protein in NC-siRNA-transfected cells, no fluorescence was detected from GFP-siRNA-transfected cells, indicating efficient knockdown of GFP by using our positive control GFP-siRNA.

Control siRNAs

3. Luciferase-siRNA

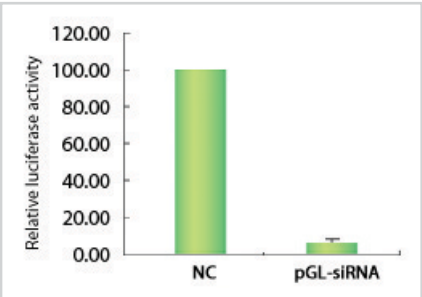


Figure 9. HeLa cells in a 6-well plate were cotransfected with 400 ng of CMV-luc plasmid and 10 nm of luciferase siRNA using lipofectamine 2000 transfection reagent. Next day, cells were harvested and assayed for luciferase activity. As shown in Fig. 9, cotransfection with our positive control luciferase siRNA led to efficient knockdown of luciferase activity (85% - 95% knockdown compared to luciferase activity of NC-siRNA-transfected cells).

Negative control

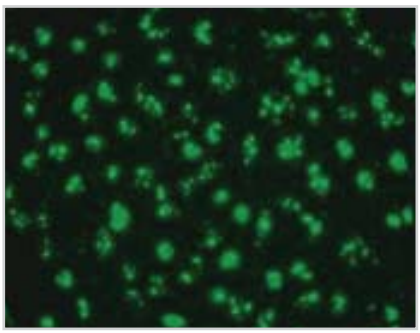


Figure 10. HeLa cells transfected with FITC-labeled siRNA (Cat No.: SN-1021) was observed by confocal microscopy. The fluorescent cells indicate that the target cells were successfully transfected with the siRNA.

■ Ordering Information

***AccuTarget*™ Positive Control siRNAs**

Cat. No.	Product Name	Purification	Guaranteed Yield
SP-1001	*AccuTarget*™ GAPDH Positive Control siRNA	BioRP	5 nmole
SP-1002	*AccuTarget*™ GAPDH Positive Control siRNA	BioRP	10 nmole
SP-1003	*AccuTarget*™ GAPDH Positive Control siRNA	BioRP	20 nmole
SP-1011	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	5 nmole
SP-1012	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	10 nmole
SP-1013	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	20 nmole
SP-2001	*AccuTarget*™ GFP Positive Control siRNA	BioRP	5 nmole
SP-2002	*AccuTarget*™ GFP Positive Control siRNA	BioRP	10 nmole
SP-2003	*AccuTarget*™ GFP Positive Control siRNA	BioRP	20 nmole
SP-2011	*AccuTarget*™ GFP Positive Control siRNA	HPLC	5 nmole
SP-2012	*AccuTarget*™ GFP Positive Control siRNA	HPLC	10 nmole
SP-2013	*AccuTarget*™ GFP Positive Control siRNA	HPLC	20 nmole
SP-3001	*AccuTarget*™ Luciferase Positive Control siRNA	BioRP	5 nmole
SP-3002	*AccuTarget*™ Luciferase Positive Control siRNA	BioRP	10 nmole
SP-3003	*AccuTarget*™ Luciferase Positive Control siRNA	BioRP	20 nmole
SP-3011	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	5 nmole
SP-3012	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	10 nmole
SP-3013	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	20 nmole

02
RNA Oligonucleotide (siRNA)

Control siRNAs

***AccuTarget*™ Negative Control siRNAs**

Cat. No.	Product Name	Purification	Guaranteed Yield
SN-1001	*AccuTarget*™ Negative Control siRNA	BioRP	5 nmole
SN-1002	*AccuTarget*™ Negative Control siRNA	BioRP	10 nmole
SN-1003	*AccuTarget*™ Negative Control siRNA	BioRP	20 nmole
SN-1011	*AccuTarget*™ Negative Control siRNA	HPLC	5 nmole
SN-1012	*AccuTarget*™ Negative Control siRNA	HPLC	10 nmole
SN-1013	*AccuTarget*™ Negative Control siRNA	HPLC	20 nmole
SN-1021	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	5 nmole
SN-1022	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	10 nmole
SN-1023	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	20 nmole

***AccuTarget*™ Control siRNA Sets**

Cat. No.	Product Name	Purification	Guaranteed Yield
SS-1001	*AccuTarget*™ GAPDH Control siRNA Set	BioRP	5 nmole positive control + 2 nmole negative control
SS-1002	*AccuTarget*™ GFP Control siRNA Set	BioRP	5 nmole positive control + 2 nmole negative control
SS-1003	*AccuTarget*™ Luciferase Control siRNA Set	BioRP	5 nmole positive control + 2 nmole negative control
SS-1011	*AccuTarget*™ GAPDH Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control
SS-1012	*AccuTarget*™ GFP Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control
SS-1013	*AccuTarget*™ Luciferase Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control

AccuTarget™ Real-Time PCR Primer Library

■ Description

The *AccuTarget*™ Human Validated Real-Time PCR Primer Library is comprised of highly specific and sensitive Real-Time PCR primer Sets that are bioinformatically designed and validated based on the human genome. The Real-Time PCR Primer Library consists of 11,154 primer Sets validated in Real-Time PCR with SYBR Green detection using *Exicycler*™ 96 and *AccuPower*® *GreenStar*™ qPCR PreMix. The Library is categorized by gene function and pathway. The Library guarantees the most specific and sensitive Real-Time PCR result (Figure. 11) when used with *AccuPower*® *GreenStar*™ qPCR PreMix (Not available in the US).

■ Features and Benefits

- In ready-to-ship format: 11,154 human genes specific primers
- All Primers pre-validated: QC tested via MALDI-TOF mass spectrometer
- Competitive pricing: Great value for your research dollar

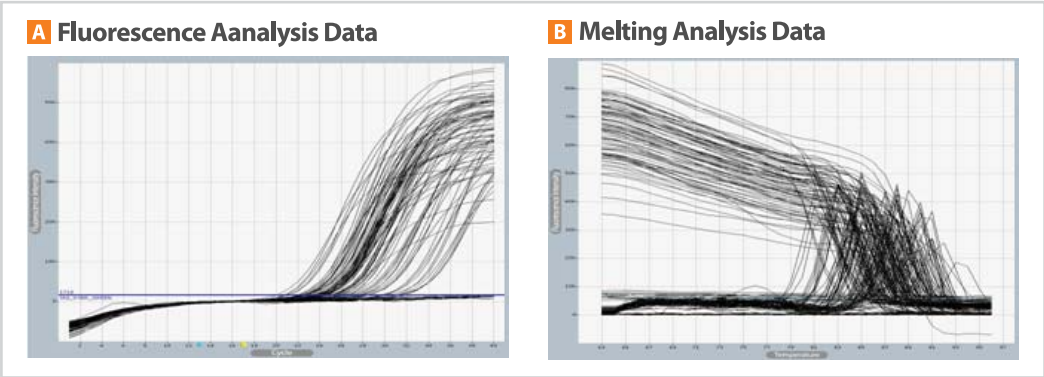


Figure 11. Real-Time PCR validation test of human Oxidoreductase using *AccuTarget*™ Human Oxidoreductase Real-Time PCR Primer Set.

■ Ordering Information

***AccuTarget*™ Real-Time PCR Primer for Individual Gene**

Cat. No.	Product Name
PHS-P01	Individual Human Real-Time PCR Primer (100 rxns)
PHS-P02	Individual Human Real-Time PCR Primer (200 rxns)

02
RNA Oligonucleotide (siRNA)

AccuTarget™ Real-Time PCR Primer Library

***AccuTarget*™ Real-Time PCR Primer Libraries**

Product Name	No. of Genes	Reactions/gene
AccuTarget™ Human Antioxidant Real-Time PCR primer Set	38	50 rxns
AccuTarget™ Human Apoptosis Real-Time PCR primer Set	277	50 rxns
AccuTarget™ Human Cancer Real-Time PCR primer Set	1,082	50 rxns
AccuTarget™ Human Caspase Real-Time PCR primer Set	35	50 rxns
AccuTarget™ Human Cell cycle Real-Time PCR primer Set	111	50 rxns
AccuTarget™ Human Cyclase Real-Time PCR primer Set	21	50 rxns
AccuTarget™ Human Cytochrome P450 Real-Time PCR primer Set	37	50 rxns
AccuTarget™ Human Deaminase Real-Time PCR primer Set	19	50 rxns
AccuTarget™ Human GPCR signaling pathway Real-Time PCR primer Set	571	50 rxns
AccuTarget™ Human Helicase Real-Time PCR primer Set	112	50 rxns
AccuTarget™ Human Isomerase Real-Time PCR primer Set	91	50 rxns
AccuTarget™ Human Kinase Real-Time PCR primer Set	673	50 rxns
AccuTarget™ Human Ligase Real-Time PCR primer Set	261	50 rxns
AccuTarget™ Human Lyase Real-Time PCR primer Set	118	50 rxns
AccuTarget™ Human Motor Real-Time PCR primer Set	111	50 rxns
AccuTarget™ Human NF-kB pathway Real-Time PCR primer Set	37	50 rxns
AccuTarget™ Human Nucleic acid binding Real-Time PCR primer Set	2,244	50 rxns
AccuTarget™ Human Oxidoreductase Real-Time PCR primer Set	502	50 rxns
AccuTarget™ Human Peptidase Real-Time PCR primer Set	463	50 rxns
AccuTarget™ Human Phosphatase Real-Time PCR primer Set	179	50 rxns
AccuTarget™ Human Receptor Real-Time PCR primer Set	1,296	50 rxns
AccuTarget™ Human Transporter Real-Time PCR primer Set	947	50 rxns
AccuTarget™ Human Tubulin Real-Time PCR primer Set	11	50 rxns
AccuTarget™ Human Ubiquitin Real-Time PCR primer Set	70	50 rxns

AccuTarget™ Human miRNAs

Overview

MicroRNAs (miRNAs) are 21-25 nucleotide (nt)-long single-stranded RNA molecules that serve as a post-transcriptional regulator of gene expression in eukaryotes. The human genome may encode over 1000 miRNAs, which bind with imperfect complementarity to their target mRNAs, generally within the 3'UTR (untranslated region), and repress protein production by destabilizing the mRNA as well as translational suppression. miRNA-mediated translational repression has an important role in wide range of biological process, including development, cell proliferation and differentiation, apoptosis and metabolism.

The biogenesis of miRNAs consists of two sequential processing events. Primary miRNA transcripts (pri-miRNAs), which contain one or multiple stem-loop hairpin structures, are mostly derived from Pol II-mediated transcription. In the first step towards the canonical miRNA maturation pathway, pri-miRNA is cleaved by the microprocessor complex, RNaseIII enzyme Drosha, to yield the pre-miRNA, a hairpin-shaped intermediate precursor ~70 nt in length. Pre-miRNAs are then exported from the nucleus to the cytoplasm by Exprotin5 protein, where another RNaseIII enzyme Dicer catalyzes the second processing event for miRNA biogenensis and liberates the mature miRNA duplexes. The mature miRNA duplexes consist of the mature miRNA strand and the miRNA* strand, which are derived from two separate arms of the hairpin stem within the miRNA precursor. The miRNA is loaded into an Argonaute-containing RNA-induced silencing complex (RISC), whereas the miRNA* strand is typically degraded. The Ago:miRNA complex then dissociates from RISC loading complex, and become the core of the RISC complex to regulate post-transcriptional gene repression of specific target miRNAs (Figure 12).

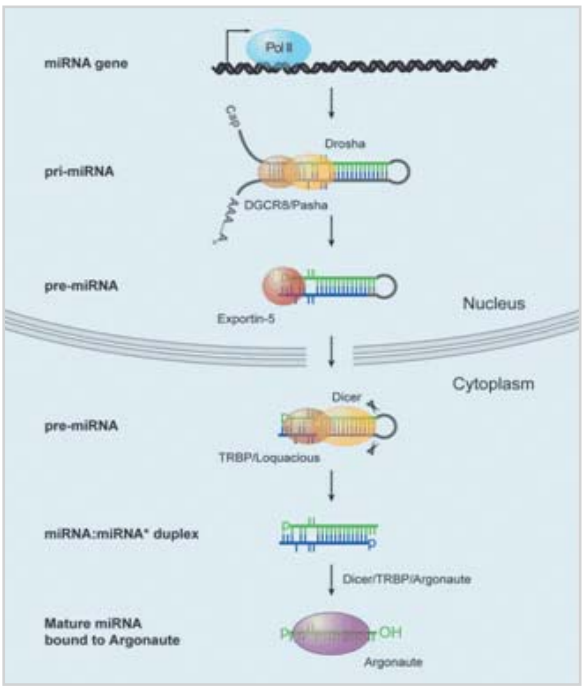


Figure 12. Biogenesis of microRNA (From Annu. Rev. Cell Dev. Biol. 2007. 23:175-205).

miRNA has provided new insights in biotechnology. Although they were discovered and recognized relatively and recently, miRNAs have been recognized as the most important gene regulators at the post-transcriptional level, and several studies indicated that miRNAs regulate the expression of more than 30% protein coding genes. The accumulating knowledge about their biogenesis, gene expression regulation mechanism and functions will add a new dimension to our understanding about the complex gene regulatory networks. Recent investigations demonstrate that miRNAs have a unique expression profiles in different cancer types at different stages and play an important role in many disease and viral infections. These results suggest that miRNAs can function as a novel biomarker for disease diagnosis and perform a new strategy for miRNA gene therapy.

02 RNA Oligonucleotide (siRNA)

AccuTarget™ Human miRNA mimics & inhibitors library

■ Description

Bioneer's *AccuTarget*™ miRNA mimics are chemically synthesized, double-stranded RNA oligonucleotides and available for 2,042 Human Mature microRNAs in the miRBase Sequence Databases. *AccuTarget*™ miRNA inhibitors are the single-stranded synthetic inhibitor targeting all human miRNAs in the miRBase Sequence Database. These miRNA mimics & inhibitors are available at 5, 10 and 20 nmole of guaranteed yield. We also offer miRNA mimics and inhibitors library Sets consisting of predesigned mimics or inhibitors at various small scales (0.25, 0.5, 1, and 2 nmole) in a 96-well plate layout to meet the unique needs of individual customers. In addition, flexible miRNA library Sets for customer- specified mimics and inhibitors are also available for the minimum order of 48.

■ Features and Benefits

Ready-to-transfect miRNA mimics behave like endogenous miRNAs and inhibitors suppress target miRNA activity to study loss-of-function effects after transfection into cells.

- Purification
  For your more demanding applications, Bioneer's automated HPLC and Bio-RP purification methods ensure high quality, high-throughput miRNA mimics and inhibitors.
- Affordable pricing
  Bioneer provides a variety of high quality miRNA products at an affordable price.
- Synthesis and QC
  Bioneer miRNA mimics and inhibitors are produced in clean room facility by fully automated high-throughput miRNA production system. Bioneer miRNA products are assessed by MALDI-TOF Mass spectrometry analysis. Mass spec. data is provided with every miRNA mimic and inhibitor. Additionally miRNA mimics are tested by gel electrophoresis to verify that both RNA strands annealed properly.

All Bioneer miRNA inhibitors are provided as single-stranded miRNA* (antisense strand of target miRNA) and all Bioneer miRNA mimics are provided as double-stranded siRNA. Each sense siRNA and an antisense siRNA are QC'ed by MALDI-TOF analysis. Every annealed double-stranded miRNA is then QC-tested using PAGE to confirm proper annealing.

■ Ordering Information

***AccuTarget*™ Custom miRNAs**

Cat. No.	Product Name	Purification	Guaranteed Yield
SMM-001	*AccuTarget*™ Human miRNA mimic	BioRP	5 nmole
SMM-002	*AccuTarget*™ Human miRNA mimic	BioRP	10 nmole
SMM-003	*AccuTarget*™ Human miRNA mimic	BioRP	20 nmole
SMI-001	*AccuTarget*™ Human miRNA inhibitor	BioRP	5 nmole
SMI-002	*AccuTarget*™ Human miRNA inhibitor	BioRP	10 nmole
SMI-003	*AccuTarget*™ Human miRNA inhibitor	BioRP	20 nmole

***AccuTarget*™ library miRNAs**

Cat. No.	Product Name	Purification	Guaranteed Yield
SML-1001	*AccuTarget*™ Human miRNA mimic	BioRP	0.25 nmole
SML-1002	*AccuTarget*™ Human miRNA mimic	BioRP	0.5 nmole
SML-1003	*AccuTarget*™ Human miRNA mimic	BioRP	1 nmole
SML-1004	*AccuTarget*™ Human miRNA mimic	BioRP	2 nmole
SML-2001	*AccuTarget*™ Human miRNA inhibitor	BioRP	0.25 nmole
SML-2002	*AccuTarget*™ Human miRNA inhibitor	BioRP	0.5 nmole
SML-2003	*AccuTarget*™ Human miRNA inhibitor	BioRP	1 nmole
SML-2004	*AccuTarget*™ Human miRNA inhibitor	BioRP	2 nmole

AccuTarget™ Human miRNA mimics & inhibitors library

■ Description

We offer *AccuTarget*™ miRNA mimic controls to optimize assay conditions for miRNA mimic function studies. Both positive and negative controls are provided for miRNA gain-of-function studies using Bioneer's *AccuTarget*™ miRNA mimics.

AccuTarget™ miRNA housekeeping Positive controls target the 3' UTR of the standard housekeeping gene 'GAPDH' and Bioneer's miRNA mimic Negative controls' sequences are based on common miRNA structure for use as negative experimental controls in human, mouse, and rat cells. The negative controls have been analyzed by BLAST against all human, mouse and rat genomic sequences and miRNA sequences in the current miRBase Database. Bioneer offers two universal negative controls for mimics. In addition, *AccuTarget*™ miRNA control Sets consisting of a Positive and two Negative miRNA controls are also available for user convenience.

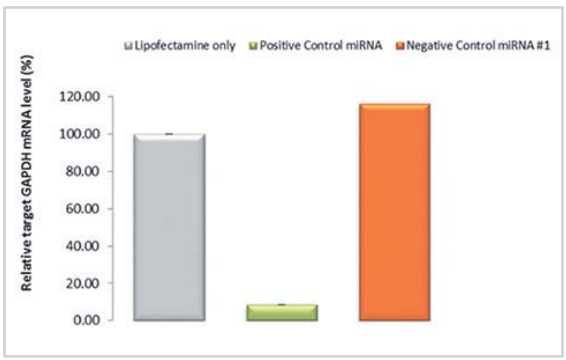


Figure 13. *AccuTarget*™ miRNA Positive and Negative controls were transfected at 20 nM using *Lipofectamine*™ RNAiMAX into HeLa cell lines and assessed for their ability to decrease target mRNA levels. Down-regulation of GAPDH was determined using the Real-Time quantitative RT-PCR at 48 hours post-transfection using Bioneer's *Exicycler*™ 96 Real-Time Quantitative Thermal Block

■ Features and Benefits

- Excellent performance
  miRNA Houskeeping Positive controls targeting GAPDH with clear read-out of mimic function (knockdown efficiency of >90 %) miRNA mimic Negative controls with minimal sequence identity with miRNAs in human, mouse and rat.
- Monitoring of transfection rate
  Fluorescence-labeled Negative controls for conveniently monitoring cellular uptake and/or transfection efficiency
- Competitive pricing
  Great value for your research dollar

02 RNA Oligonucleotide (siRNA)

AccuTarget™ Human miRNA mimic controls

■ Ordering Information

***AccuTarget*™ Control miRNAs**

Cat. No.	Product Name	Purification	Guaranteed Yield
SMC-1001	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	BioRP	5 nmole
SMC-1002	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	BioRP	10 nmole
SMC-1003	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	BioRP	20 nmole
SMC-2001	*AccuTarget*™ miRNA mimic Negative control #1	BioRP	5 nmole
SMC-2002	*AccuTarget*™ miRNA mimic Negative control #1	BioRP	10 nmole
SMC-2003	*AccuTarget*™ miRNA mimic Negative control #1	BioRP	20 nmole
SMC-3001	*AccuTarget*™ miRNA mimic Negative control #2	BioRP	5 nmole
SMC-3002	*AccuTarget*™ miRNA mimic Negative control #2	BioRP	10 nmole
SMC-3003	*AccuTarget*™ miRNA mimic Negative control #2	BioRP	20 nmole
SMC-4001	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	BioRP	5 nmole
SMC-4002	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	BioRP	10 nmole
SMC-4003	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	BioRP	20 nmole
SMC-5001	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	BioRP	5 nmole
SMC-5002	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	BioRP	10 nmole
SMC-5003	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	BioRP	20 nmole

RNA Oligonucleotide FAQs

1. What form will my order be in?

For Genome-Wide Predesigned siRNAs, Validated siRNAs, siRNA Libraries and Control siRNAs, both the sense and antisense strands are synthesized at equimolar concentrations, verified via MALDI-TOF, then annealed and delivered in duplexed, lyophilized form. You may reconstitute the siRNA with a buffer of your choice or with ultrapure water that we provide with every order. We recommend reconstituting to 100 μm. For Custom siRNA orders, the order is processed by the same method as above. We recommend 50 μM reconstitution for Custom siRNA orders. When ordering Custom siRNA you must select the "Annealing Service" to receive your order in annealed form. If you choose not to use our annealing service, you can use 1X annealing buffer and follow the annealing protocol included with your Custom siRNA order.

2. I want to conduct an *in vitro* experiment. What scale should I choose? What purification should I select?

With a 10 nmole scale siRNA order, you can transfect one hundred (100) wells in 6-well plates at 100 nm per transfection. Unless you are planning to conduct an *in vivo* experiment, the Bio-RP Purification will yield outstanding results. We recommend HPLC purification for *in vivo* experimental use.

3. The Genome-wide predesigned siRNA didn't work like I expected. What do I do?

Our Genome-wide predesigned siRNAs provide three candidates per target gene. In order to request more than four candidates, order via Custom siRNA request. The siRNA sequences can be verified only after the order has been submitted. If you would like to compare the sequences with publications or to modify your order, please email us at sirna-support@bioneer.co.kr or call us at +82-42-930-8777.

4. Do phosphate groups present on the 5' or 3' ends of the synthesized siRNA?

Unless explicitly stated, the 5' and 3' ends are capped with -OH groups. Therefore, to order 5' phosphate-capped siRNAs, you must request for 5' phosphorylation modification.

5. How do I store my siRNAs and how long can I keep them?

siRNAs can normally be kept stable at -20°C for over 1 year. The lyophilized form is especially stable and has a longer shelf-life. Although dissolved siRNAs is stable, contamination of the reconstitution solution with RNase will degrade the product. Also, repeated freeze-thaw cycles accelerate the degradation process. Therefore, we recommend that after you receive the siRNA stock, you reconstitute it and make several aliquots to avoid such freeze-thawing. Because the phosphodiester bonds of the RNA can be broken under high pH conditions, we ask you to take caution, and recommend reconstituting in ultrapure water provided.

6. How do I store my fluorescent dye modified siRNA?

Photobleaching may occur if the fluorescent dye modified siRNA is exposed to light for prolonged periods of time. Therefore we recommend that you store such siRNAs in a dark container, and store that container in a dark place.

7. Can I know how many ng the synthesized product is?

Normally, we will fulfill an order with a guaranteed nmole amount, and the synthesis report will also report the final amount in nmoles. If you must have the ng amount to calculate for an experiment, you can convert from nmole to ng by using the formula below. We make it easy for you by giving you the molecular weight of the siRNA sequence in the report. Molecular Weight (g) X mole (nmole) = Mass of siRNA (ng).

8. This is my first siRNA experiment. How do I Set my experimental conditions?

One of the most important factors in a siRNA experiment is the assessment of whether the siRNA gets delivered into the cell. Bioneer offers positive controls that can easily indicate whether the siRNA is being delivered successfully.

9. What are some precautions for a siRNA experiment?

Firstly, because not all siRNAs will knock-down the target gene with identical efficiency, you should try 2-3 different sequences to find the best siRNA. Secondly, to make sure that the knock-down affects downstream protein expression, miRNA levels should also be measured. Thirdly, verify the knock-down phenotype by using another siRNA designed for the same target gene and show that the same phenotype appears.

02
RNA Oligonucleotide (siRNA)

RNA Oligonucleotide FAQs

10. How do I use 10 nmole of siRNA to transfect cells at 100 nM?

This is a source of confusion for many people. In order to transfect a single well with 100 nm siRNA where the transfection volume is 1 ml, you need 100 pmole of siRNA. 2 µl of 50 µM (50 pmole/µl) stock siRNA solution in 1 ml will yield 100 pmole. If you were to order 10 nmole of a siRNA, it will be sufficient to transfect 100 wells at 100 nm (100 pmole/ml).

11. How do I verify my siRNA transfection efficiency?

You can easily verify the transfection efficiency by transfecting your cells with NC-FITC and observing the cells with a fluorescence microscope. The NC-FITC can also be used as a test reagent to optimize the transfection concentrations of both the siRNA and the transfection reagent.

12. How do I verify the siRNA knockdown efficiency?

The siRNA knockdown efficiency can be verified through various techniques including qPCR, Northern Blot, Western Blot etc.

User Protocol

Dilution Protocol

1. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the siRNA pellet is located at the bottom of the tube.

2. Dissolve siRNAs to a convenient stock concentration using the recommended volume of DEPC-DW (or RNase-free water) shown in Table 1.

3. Pipette the solution up and down 3-5 times (or vortex briefly).

4. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the solution is collected at the bottom of the tube.

5. Aliquot the siRNAs into small volumes and store at -20°C. siRNA is stable for 1 year under the specified storage condition. For best results, limit freeze-thaw events for each tube no more than five.

Table 1. Recommended siRNA resuspension volumes and concentrations

siRNA Amount (nmol)	DEPC-DW volume(μl) for desired final concentration	
	100 μm stock	20 μm stock
10	100	500
20	200	1000
50	500	Exceeds tube volume
100	1000	

Transfection Protocol

* We use the *Lipofectamine*™ RNAiMAX (invitrogen; Cat.No. 13778) and HeLa cell for transfection procedure.

* This protocol is fixed at 6-well plate *in vitro* culture condition (if you want to change this condition, you have to consider the relative surface area (table 2) and invitrogen protocol, when you are seeding the cells into the culture dish).

1. One day (24 hours) before transfection, plate 3.0 X 10^5 HeLa cells in each well with 2.5 ml of growth medium without antibiotics such that they will be 50-60% confluent at the time of transfection.

2. Remove the growth medium from the 6-well plate before transfection. And add the 500 μl fresh growth medium without serum in each well.

3. For each well to be transfected, prepare siRNA duplex-*Lipofectamine*™ RNAiMAX complexes as follows.

3-1. Dilute siRNA duplex (making final concentration as 5 nm-100 nm) in 250 μl growth medium (or Opti-MEM® I Reduced Serum medium) without serum. Mix gently by vortex.

3-2. Mix *Lipofectamine*™ RNAiMAX gently before use, then dilute 3.5 μl in 250 μl medium (or Opti-MEM® I Reduced Serum medium) without serum. Incubate this solution 5 minutes at room temperature.

3-3. Combine the diluted siRNA duplex with the diluted *Lipofectamine*™ RNAiMAX. Mix and incubate for 20 minutes at room temperature.

4. Add the mixture to each well containing HeLa cells, which result 1 ml as total volume. Mix gently by hand rocking the plate back and forth.

5. Incubate the cells for 5-6 hours at 37°C in CO_2 incubator.

6. Change the medium with fresh one containing serum and incubate the cells 24-48 hours until you are ready to assay for gene knockdown.

Table 2. The relative surface area of *in vitro* cell culture dish and culture media volume

Culture Vessel	Relative surface area	Volume of plating medium
96-well	0.2	100 μl
48-well	0.4	200 μl
24-well	1	500 μl
6-well	5	2.5 ml
60 mm	10	5 ml
100 mm	30	10 ml

03 DNA/RNA Synthesis Reagents

Bioneer Corporation

DNA/RNA Synthesis Reagents

DNA/RNA Synthesis Reagents

Oligonucleotide synthesis, siRNA and more

Overview

Bioneer has long been a leading manufacturer of phosphoramidites and oligonucleotides and has extensive expertise and experience in these areas. Bioneer is an ISO 9001:2000-certified company with all products being manufactured under strict ISO 9001:2000 standards.

■ Process

Bioneer has four 6,000 L capacity reactors for synthesizing four different nucleosides. All nucleoside phosphoramidites are manufactured in 6,000 L reactors. Each reactor has a capacity of up to 50 kg per batch. Per annum total manufacturing capacity for nucleoside phosphoramidites is approximately 4 tons. The resulting DNA nucleoside phosphoramidites are purified with column chromatography for purities of ≥99%. Four Sets of 400 L volume columns are utilized for the separation of each different nucleoside phosphoramidite. The purified/crystallized nucleoside phosphoramidites are finally filtered and dried to yield the final product in powder form. The process is carefully moisture-controlled to maintain less than 40 ppm water content after being dissolved in acetonitrile, the solvent for oligonucleotide synthesis.

03 DNA/RNA Synthesis Reagents

DNA Phosphoramidites

Features and Benefits

- Amine functions are protected by protecting groups {dA (Bz), dC (Bz), dC (Ac), dG (Ib)}.
- The recommended procedure for cleavage and deprotection for standard oligonucleotide synthesis is treatment with concentrated ammonia for 8 hours at 55°C or by the use of methylamine gas.
- The purities of manufactured nucleoside phosphoramidites are determined by 31P-NMR and RP-HPLC analysis and controlled to be greater than 99%.
- All reagents are conveniently packaged for ABI instruments. Expedite- and Mermade-compatible vials are also available upon request.

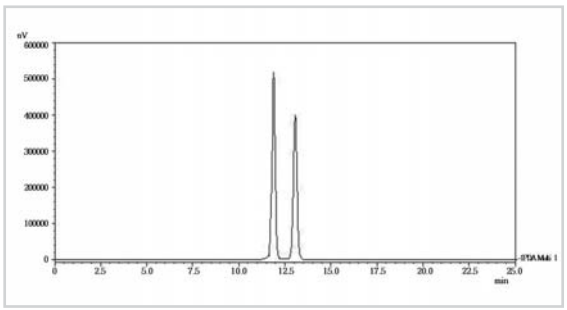


Figure 1. dA(Bz)-Phosphoramidite HPLC Data RP- HPLC : ≥ 99%

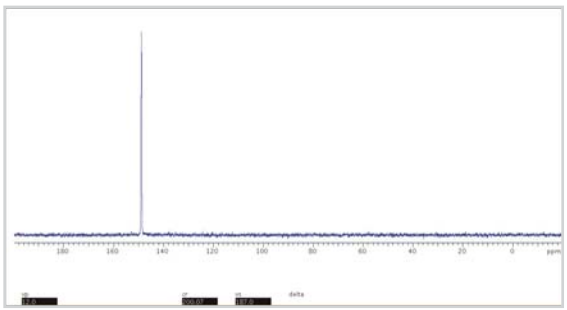
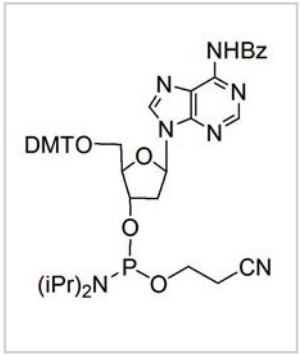


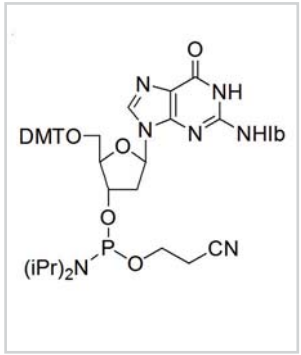
Figure 2. dA(Bz)-Phosphoramidite ^{31}P-NMR Data ^{31}P-NMR : ≥ 99%

Ordering Information

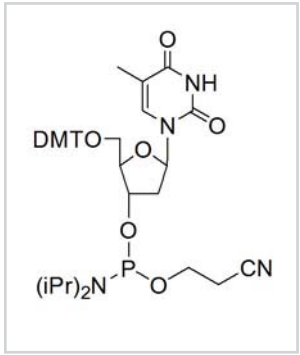
Cat. No.	Product Name	Packing
M-1001-1	dA(Bz)-CE phosphoramidite	1 g
M-1001-2		20 g
M-1002-1	dC(Bz)-CE phosphoramidite	1 g
M-1002-2		20 g
M-1003-1	dG(Ib)-CE phosphoramidite	1 g
M-1003-2		20 g
M-1004-1	dT-CE phosphoramidite	1 g
M-1004-2		20 g
M-1005-1	dC(Ac)-CE phosphoramidite	1 g
M-1005-2		20 g



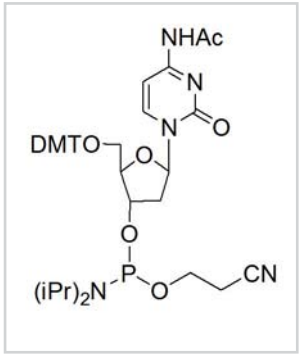
dA(Bz)-CE phosphoramidite



dG(Ib)-CE phosphoramidite



dT-CE phosphoramidite



dC(Ac)-CE phosphoramidite

RNA Phosphoramidites

Features and Benefits

- Amine groups are protected by protecting groups {rA (Bz), rC (Ac) rG (Ib)}.
- Cleavage and deprotection procedures for RNA synthesis are similar to DNA synthesis, with an additional step to remove the 2'-OH protecting group.
- The 2'-OH group is protected by a tert-butyldimethylsilyl (TBDMS) group to prevent derivatization and degradation during the synthesis cycle.
- Standard RNA phosphoramidites provide excellent coupling efficiency when used together with either ETT or BTT as an activator.
- The purities of manufactured nucleoside phosphoramidites are quality controlled via RP-HPLC/31P-NMR analysis. (RP-HPLC : ≥ 99%, 31P-NMR : ≥ 99%)
- All reagents are conveniently packaged for ABI instruments. Expedite-and Mermade-compatible vials are also available upon request.

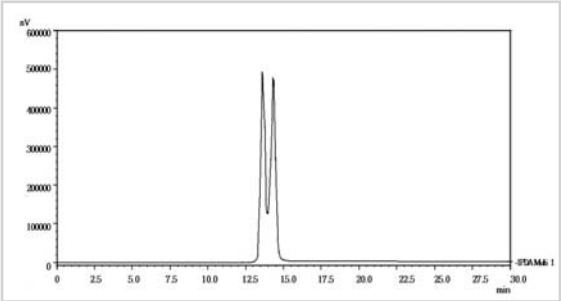


Figure 1. rC(Ac)-Phosphoramidite HPLC Data RP- HPLC : ≥ 99%

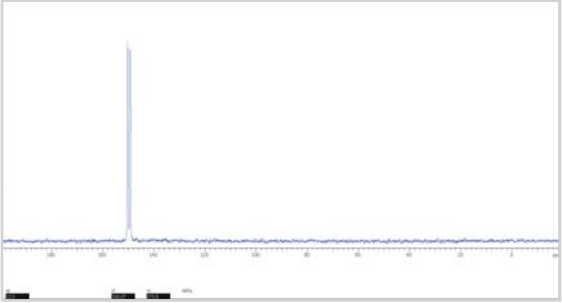
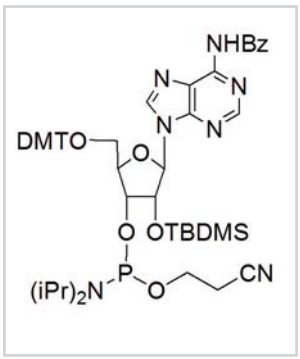


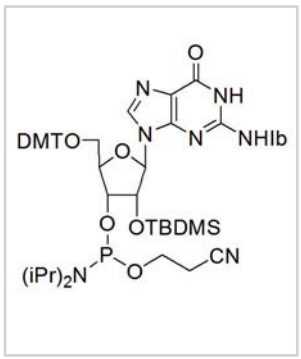
Figure 2. rC(Ac)-Phosphoramidite 31P-NMR Data 31P-NMR : ≥ 99%

Ordering Information

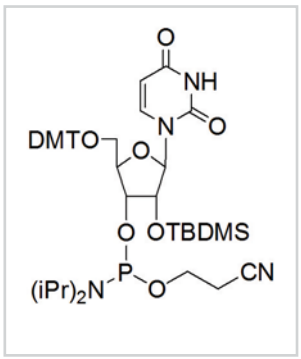
Cat. No.	Product Name	Packing
M-1101-1	rA(Bz)-CE phosphoramidite	0.5 g
M-1101-2		10 g
M-1103-1	rG(Ib)-CE phosphoramidite	0.5 g
M-1103-2		10 g
M-1104-1	rU-CE phosphoramidite	0.5 g
M-1104-2		10 g
M-1105-1	rC(Ac)-CE phosphoramidite	0.5 g
M-1105-2		10 g



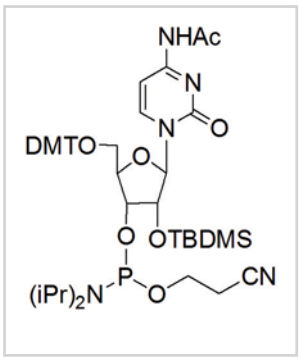
rA(Bz)-CE phosphoramidite



rG(Ib)-CE phosphoramidite



rU-CE phosphoramidite



rC(Ac)-CE phosphoramidite

03 DNA/RNA Synthesis Reagents

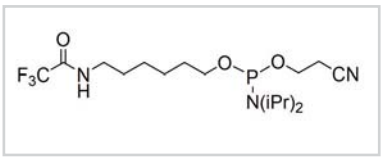
Amino linker phosphoramidites

Features and Benefits

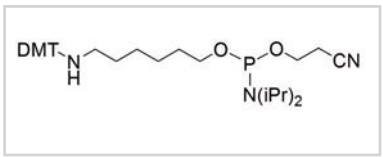
- Amino linker products can be coupled with standard synthesis protocols, such as DNA monomer phosphoramidite coupling protocols.
- The primary amino group can be used for subsequent conjugation of a variety of reporter dyes or labels.
- The base-labile TFA-group is easily cleaved with concentrated ammonia during the cleavage and deprotection steps. Additional deprotection steps are not necessary.
- The DMT-group can be cleaved more conveniently than the MMT-group on the synthesis instrument with an acidic deblocking solution to enable on-support labeling protocols.
- The 5'-DMT amino linker can be treated with normal deprotection and cleavage methods.

Ordering Information

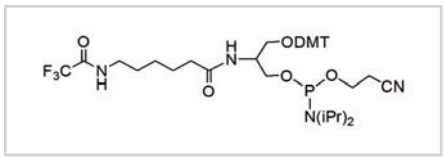
Cat. No.	Product Name	Packing
M-2001-1	5′-TFA Amine linker	0.25 g
M-2001-2		0.1 mmol
M-2002-1	5′-DMT Amine linker	0.25 g
M-2002-2		0.1 mmol
M-2003-1	3′-TFA Amine linker	0.25 g
M-2003-2		0.1 mmol



5′-TFA Amine linker



5′-DMT Amine linker



3′-TFA Amine linker

Spacer phosphoramidites

Features and Benefits

- Spacer phosphoramidites are used to place spacer arms into oligonucleotides. C3, C6 and C12 spacer phosphoramidites contain an aliphatic linker which can be added to oligos requiring long spacer arms. In general, spacer phosphoramidites are used during oligonucleotide synthesis to introduce a spacer into the sequence, bridging sections of an oligonucleotide.
- C6, C16-6, C18-6 disulfide phosphoramidites contain a disulfide linker which can be added to oligos requiring disulfide spacer arms.
- 12, 18 atom spacer phosphoramidites contain an ethylene glycol linker which can be added to oligos requiring ethylene glycol spacer arms.
- Abasic and dSpacers are used to introduce a stable abasic site within an oligonucleotide.
- Hexadecane and octadecane phosphoramidites are hydrophobic.

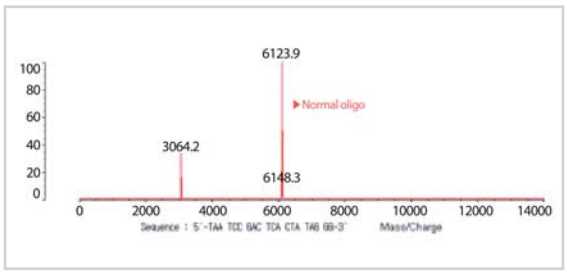


Figure 1. Normal Oligo MALDI-TOF Data

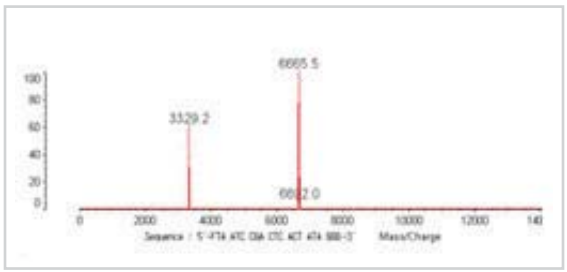


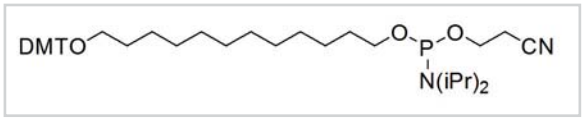
Figure 2. 5'-Fluorescein Oligo MALDI-TOF Data

Ordering Information

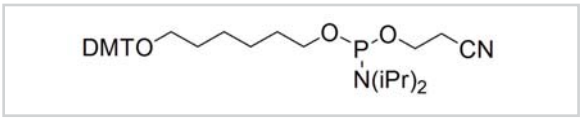
Cat. No.	Product Name	Packing
M-2011-1	C12 Spacer phosphoramidite	0.25 g
M-2011-2		0.1 mmol
M-2012-1	C6 Spacer phosphoramidite	0.25 g
M-2012-2		0.1 mmol
M-2013-1	C3 Spacer phosphoramidite	0.25 g
M-2013-2		0.1 mmol
M-2017-1	C6 Disulfide phosphoramidite	0.25 g
M-2017-2		0.1 mmol
M-2019-1	12 Atom Spacer phosphoramidite	0.25 g
M-2019-2		0.1 mmol
M-2020-1	18 Atom Spacer phosphoramidite	0.25 g
M-2020-2		0.1 mmol
M-2024-1	Abasic phosphoramidite	0.25 g
M-2024-2		0.1 mmol
M-2025-1	dSpacer phosphoramidite	0.25 g
M-2025-2		0.1 mmol
M-2027-1	Hexadecane phosphoramidite	0.25 g
M-2027-2		0.1 mmol
M-2028-1	Octadecane phosphoramidite	0.25 g
M-2028-2		0.1 mmol
M-2029-1	C16-6 Disulfide phosphoramidite	0.25 g
M-2029-2		0.1 mmol
M-2030-1	C18-6 Disulfide phosphoramidite	0.25 g
M-2030-2		0.1 mmol

03
DNA/RNA Synthesis Reagents

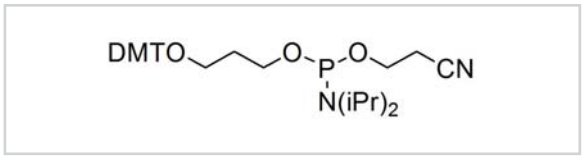
Spacer phosphoramidites



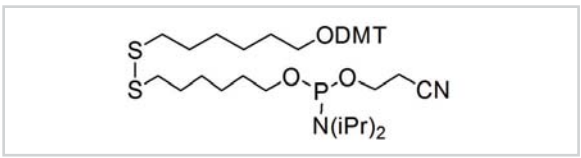
C12 Spacer phosphoramidite



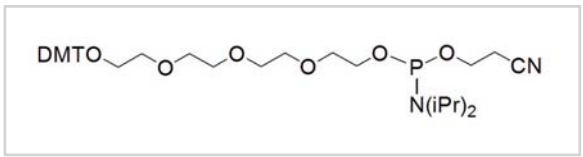
C6 Spacer phosphoramidite



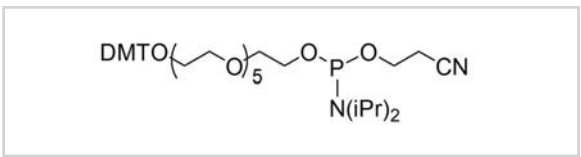
C3 Spacer phosphoramidite



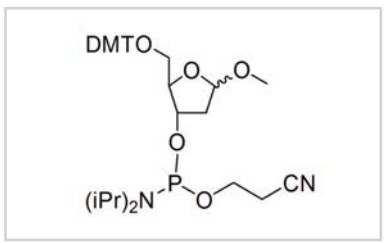
C6 Disulfide phosphoramidite



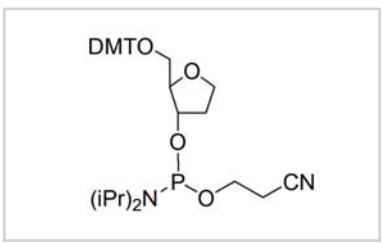
12 Atom Spacer phosphoramidite



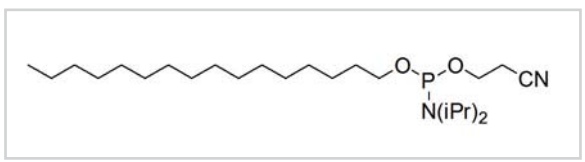
18 Atom Spacer phosphoramidite



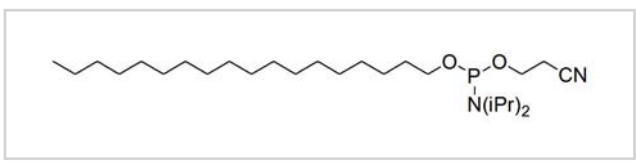
Abasic phosphoramidite



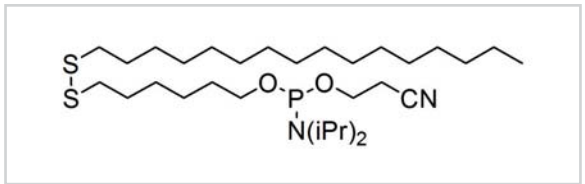
dSpacer phosphoramidite



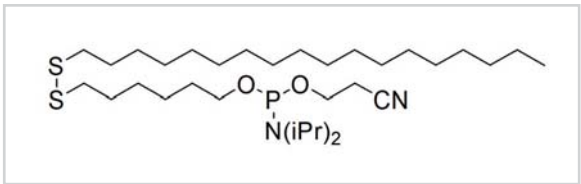
Hexadecane phosphoramidite



Octadecane phosphoramidite



C16-6 Disulfide phosphoramidite



C18-6 Disulfide phosphoramidite

Labeling Modifier

Features and Benefits

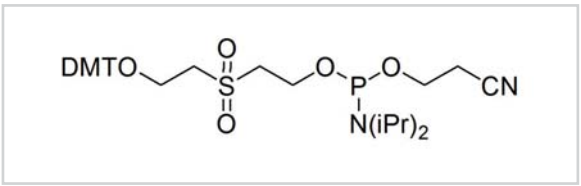
- Chemical phosphorylation reagents are most commonly used to phosphorylate the 5'-terminus of an oligonucleotide.
- 5'-Biotin phosphoramidite is a reagent used to directly incorporate a biotin label at the 5'-terminus during solid phase oligonucleotide synthesis. The DMT (dimethoxytrityl) group on the biotin moiety can be removed during synthesis to measure coupling efficiency or retained on the oligonucleotide to facilitate RP or cartridge purification. The 5'-biotin label is attached to the oligonucleotide through a 6-carbon atom (aminohexanol) spacer arm.
- Oligonucleotide 5' end labeling can be used for fluorescent probe synthesis. Oligonucleotides modified with fluorescein derivatives can be used for genomic research, molecular diagnostics and molecular biology.
- dI-phosphoramidites can perform as universal bases as they hybridize with any of the four natural bases.
- 2'-F-RNA oligonucleotides adopt an A-form helix on hybridization to a target. Aptamers composed of 2'-F-RNA bind targets with higher affinity and are more resistant to nucleases compared to RNA aptamers. 2'-F-RNA can be effectively used in siRNA applications, especially in RNA interference for the specific silencing of genes in cells and *in vivo*. Bioneer provides two monomer types: 2'-F-rC phosphoramidite and 2'-F- rU phosphoramidite.
- 2'-OMe oligo ribonuleotides are extremely useful reagents for a variety of molecular biology applications. 2'-OMe-RNA is chemically more stable than either DNA or RNA and is resistant to degradation by RNA- or DNA- specific nucleases. Bioneer provides two monomers: 2'-OMe-rU-phosphoramidite and 2'-OMe-rC phosphoramidite.
- The internal-dT amine phosphoramidite is a sequence modifier (amino-modifier) which can be Set in the target position during the oligonucleotide synthesis process. After oligo synthesis and the deprotection process have been performed, the internal- dT amine can form a primary amine on the phosphoramidite. This primary amine can be bind with other target materials that have a NHS-ester moiety.

03 DNA/RNA Synthesis Reagents

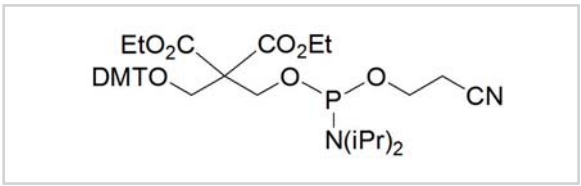
Labeling Modifier

Ordering Information

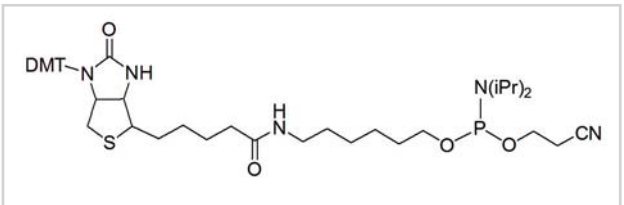
Cat. No.	Product Name	Packing
M-2004-1	Phosphorylation reagent	0.25 g
M-2004-2		0.1 mmol
M-2005-1	Phosphorylation reagent II	0.25 g
M-2005-2		0.1 mmol
M-2006-1	5′-Biotin phosphoramidite	0.25 g
M-2006-2		0.1 mmol
M-2008-1	5′-Fluorescein phosphoramidite	0.25 g
M-2008-2		0.1 mmol
M-2009-1	5′-Tamra phosphoramidite	0.25 g
M-2009-2		0.1 mmol
M-2010-1	dI phosphoramidite	0.25 g
M-2010-2		0.1 mmol
M-2021-1	dU phosphoramidite	0.25 g
M-2021-2		0.1 mmol
M-2022-1	2′-F-rC(Bz) phosphoramidite	0.25 g
M-2022-2		0.1 mmol
M-2036-1	2′-F-rC(Ac) phosphoramidite	0.25 g
M-2036-2		0.1 mmol
M-2023-1	2′-F-rU phosphoramidite	0.25 g
M-2023-2		0.1 mmol
M-2031-1	Internal-dT amine phosphoramidite	0.25 g
M-2031-2		0.1 mmol
M-2032-1	2′-OMe-rU-phosphoramidite	0.25 g
M-2032-2		0.1 mmol
M-2033-1	2′-OMe-rC(Ac)-phosphoramidite	0.25 g
M-2033-2		0.1 mmol



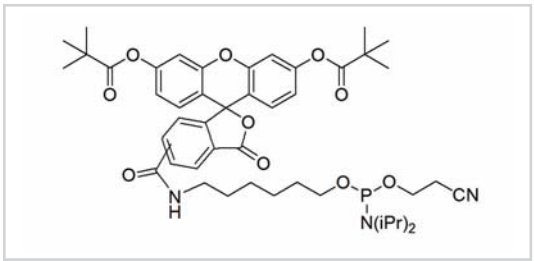
Phosphorylation reagent



Phosphorylation reagent II

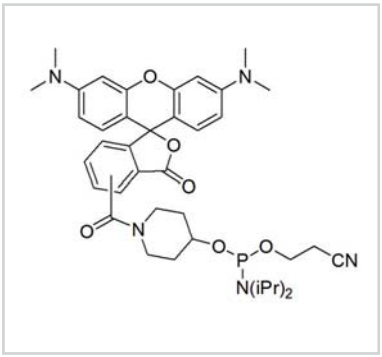


5′-Biotin phosphoramidite

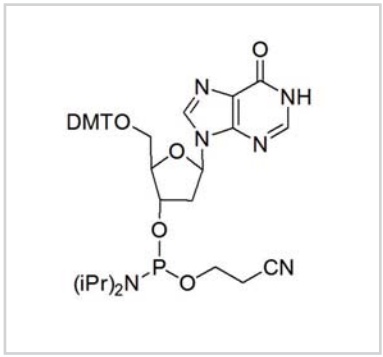


5′-Fluorescein phosphoramidite

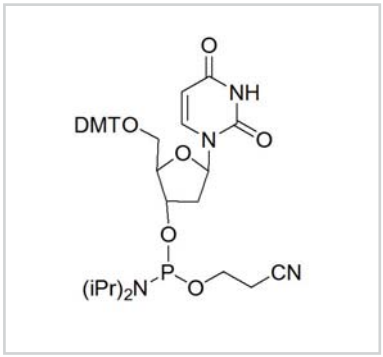
Labeling Modifier



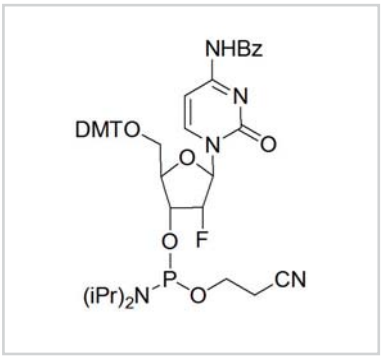
5′-Tamra phosphoramidite



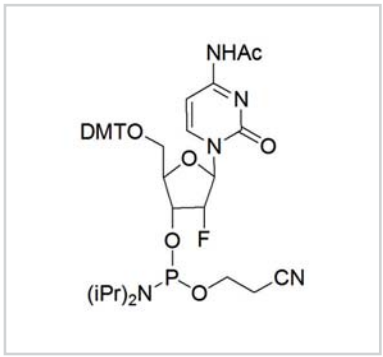
dI phosphoramidite



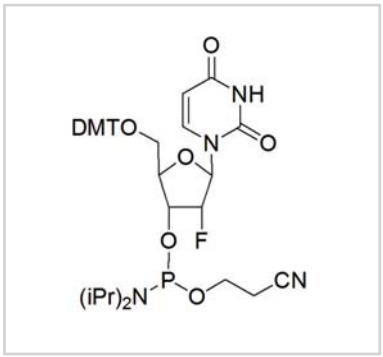
dU phosphoramidite



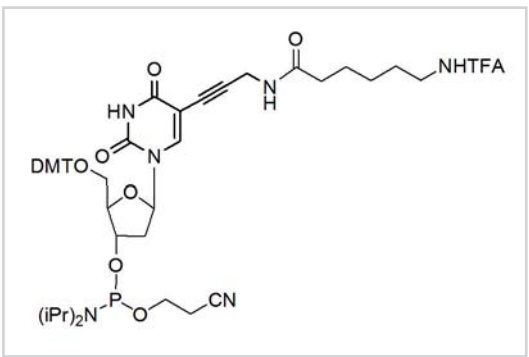
2′-F-rC(Bz) phosphoramidite



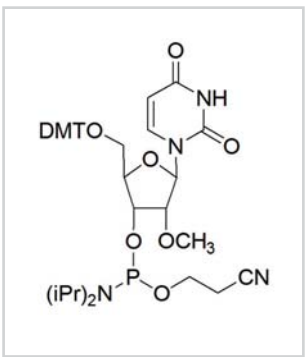
2′-F-rC(Ac) phosphoramidite



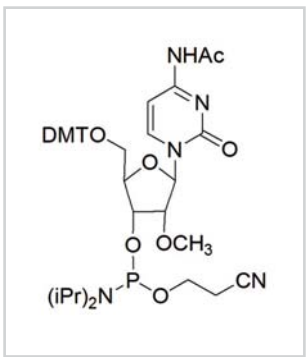
2′-F-rU phosphoramidite



Internal-dT amine phosphoramidite



2′-OMe-rU-phosphoramidite



2′-OMe-rC(Ac)-phosphoramidite

03 DNA/RNA Synthesis Reagents

CPGs for oligo synthesis

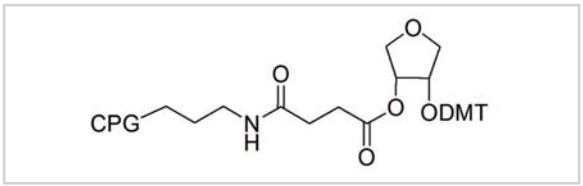
Features and Benefits

- Controlled Pore Glass (CPG) is the gold standard for solid-phase oligonucleotide synthesis, with features such as high surface area, tightly controlled pore size and chemical inertness. A variety of CPGs for oligonucleotide synthesis is available and can be purchased for standard synthesis sizes or for long-mer synthesis.
- 3'-Amine-CPG is used to place a 3'-amine end into oligonucloetides. It can be used to bind with other target materials with a NHS-ester moiety.

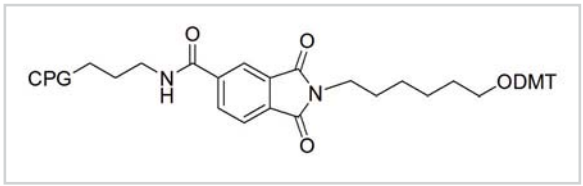
Ordering Information

Cat. No.	Product Name	Packing
M-3001-1	Universal Solid Support (STD, 1000 Å)	1 g
M-3001-2		10 g
M-3003-1	Universal Solid Support (Long, 2000 Å)	1 g
M-3003-2		10 g
M-3004-1	dA(Bz)-CPG (1000 Å)	1 g
M-3004-2		10 g
M-3005-1	dC(Bz)-CPG (1000 Å)	1 g
M-3005-2		10 g
M-3006-1	dG(Ib)-CPG (1000 Å)	1 g
M-3006-2		10 g
M-3007-1	dT-CPG (1000 Å)	1 g
M-3007-2		10 g
M-3013-1	3'-Aminio CPG (STD, 1000 Å)	1 g
M-3013-2		10 g

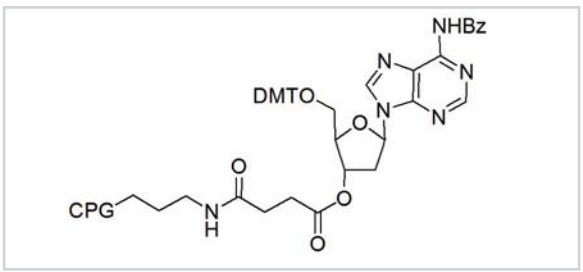
CPGs for oligo synthesis



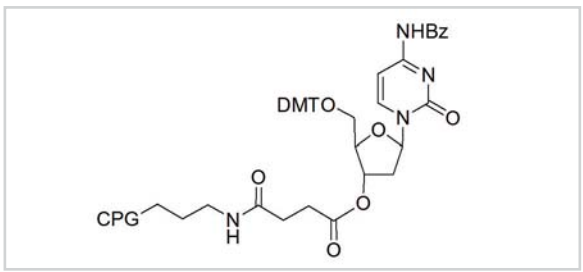
Universal Solid Support



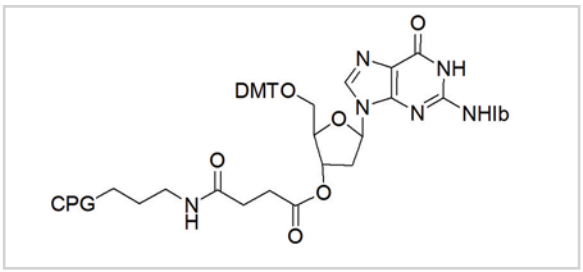
3'-Aminio CPG



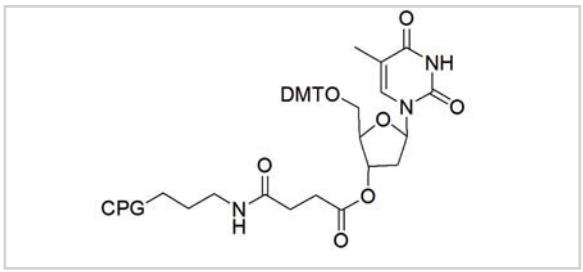
dA(Bz)-CPG



dC(Bz)-CPG



dG(ib)-CPG



dC(Bz)-CPG

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03
DNA/RNA
Synthesis Reagents