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INSIDE



# VERSATILE APPLICATIONS OF THE COPPER(I)-CATALYZED CLICK CHEMISTRY

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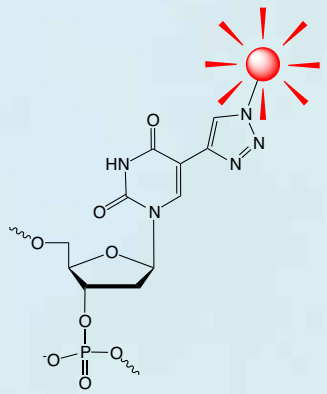
*Note: Products of baseclick are patent protected and available from Glen Research Corporation, 22825 Davis Drive, Sterling, VA 20164, USA, email: support@glenresearch.com, in collaboration with baseclick.*

FLUOROPHORE AZIDE CLICKED TO EdU



## INTRODUCTION

Since the discovery of the copper(I)-catalyzed reaction between azide and alkyne (CuAAC)-moiety in 2002,[1] its unique characteristics, high yield and mild reaction conditions, have made it one of the most prominent if not "the" click reaction. Although the purpose of this reaction, joining two molecules, is very simple, the plethora of applications that make use of this efficient chemistry demonstrates that only creativity and imagination may be the main limits for its application. Using CuAAC for the modification of oligonucleotides has been particularly fruitful [2] and is the foundation of the company *baseclick*. The following applications exemplify the broad scope of the method.

## APPLICATIONS:

### *1) Click Chemistry for Cell Proliferation Detection*

The detection of cell proliferation is of utmost importance for cell imaging, the diagnosis of genotoxicity, the evaluation of anti-cancer drugs, to name just a few. In order to detect DNA Synthesis of dividing cells, an alkyne bearing thymidine analogue, EdU (5-Ethynyl-2'-deoxyuridine), is incorporated into DNA during cell division.[3] For analysis, the fluorophore azide is subsequently "clicked" to the sample and detected using various methods (see Figure 1, Page 2). Only cells which have newly synthesized DNA are detected by microscopy, fluorescence-activated cell sorting (FACS) techniques or high throughput screening (HTS). Notably, the EdU method has high selectivity, sensitivity and reproducibility. Moreover, it allows nearly unrestricted choice of the fluorescent label.

Currently, a combined approach by baseclick and the group of Prof. Thomas Carell (LMU Munich) aims for further improved sensitivity of the technology.

### *2) DNA nanotechnology*

DNA nanotechnology is an emerging research field. The user-defined self-assembly of nucleic acids to construct DNA nanostructures of various shapes opens new DNA applications[4], e.g., as drug delivery tools, a platform for single molecule reaction, molecular machines and many more. Unfortunately, these complex structures require high salt concentrations to maintain correct folding.

By applying click chemistry to DNA nanotechnology, baseclick has created very stable nanostructures.[5] A DNA nanotube (24 nm in

*(Continued on Page 2)*

(Continued from Front Page)

length) was designed from a set of doubly modified oligonucleotides (alkyne and azide functionality on each oligonucleotide). After folding of the nanotube, the strands were interlocked at the ends by CuAAC (Figure 2). The nanotube is so robust that it remains intact even under denaturing conditions and in the presence of nucleases.

### *3) Next Generation DNA-FISH and mRNA FISH Probes*

Fluorescence *in situ* hybridization (FISH) is a molecular cytogenetic technique that enables the detection and localization of specific DNA or RNA sequences on chromosomes and cell compartments (target). The principle of this method is to hybridize labelled single stranded nucleic acid molecules such as DNA and RNA (probe) to complementary target sequences.

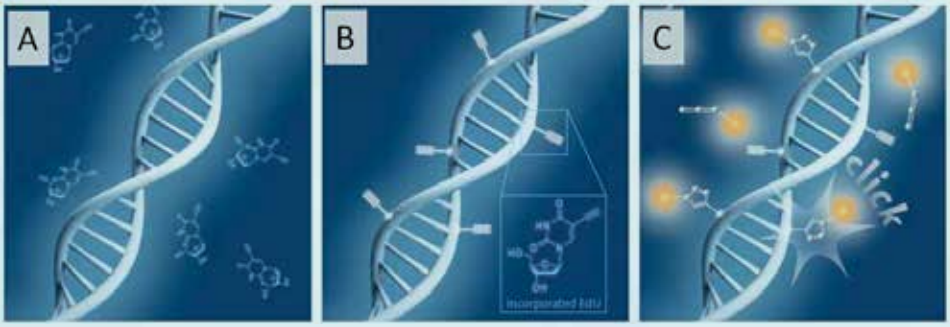
Baseclick FISH probes are oligonucleotides containing two or three reporter molecules attached by CuAAC for improved signal intensity.[6] These FISH probes are hybridized with the target and detected subsequently by microscopy. A strategy where clickable FISH probes are hybridized with the target and labelled afterwards is also feasible. This so-called post-hybridization click condition allows the use of sensitive reporter molecules that are mostly not available to standard FISH probes. Baseclick FISH probes display a high signal-to-noise ratio, possess high labelling rates and have an easy-to-handle protocol (Figure 3).

At the moment, a combined effort of baseclick and the Carell group at the LMU Munich is aiming for further improved sensitivity of the method using dendrimer structures for increased fluorescence of the probe.

### *4) CuAAC for Preparation of Bispecific Aptamers*

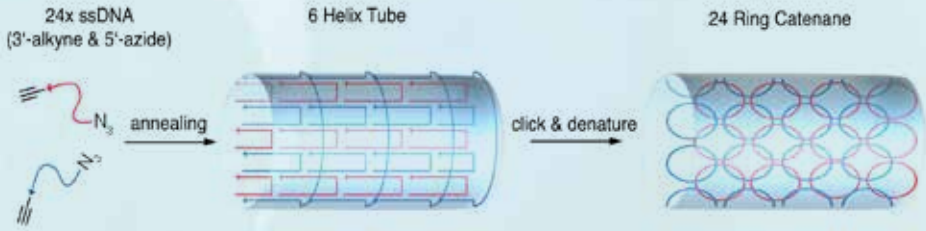
Aptamers are oligonucleotide sequences (DNA or RNA) which have outstanding potential for applications in biosensing, bioimaging and cancer therapy. They specifically bind another molecule (e.g., nucleic acids, proteins, small organic compounds) in a manner similar to antibodies. Aptamers can be prepared by solid-phase synthesis. Thus they are commercially available and can be easily modified site-specifically in contrast to antibodies. Using the CuAAC technology, baseclick has prepared aptamers with two different binding specificities combined in a single aptamer molecule by efficiently joining two aptamers. These aptamers have similar applications as bi-specific antibodies and thus could bring together two different cells (e.g., cancer cell and T cell) and induce targeted cancer cell destruction.

**FIGURE 1: SCHEMATIC ILLUSTRATION OF THE CLICK CHEMISTRY BASED EdU CELL PROLIFERATION ASSAY**



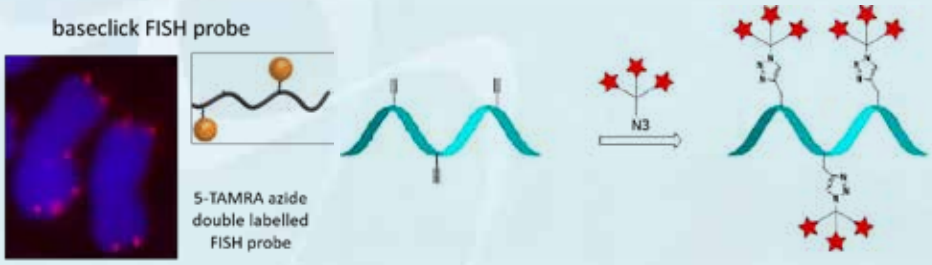

A) Incubation of cells with EdU. B) EdU is incorporated during active DNA synthesis. C) Detection of proliferating cells *via* click chemistry, whereby a variety of different fluorescent dye azides can be used.

**FIGURE 2: SCHEMATIC REPRESENTATION OF A DNA TUBE**




Schematic representation of a DNA tube consisting of 24 (3'-alkyne, 5'-azide)-modified oligonucleotides. The pre-organized position of the click reactive groups within the tube is achieved by strand hybridization. After addition of a click catalyst, the intramolecular reaction is carried out yielding a DNA catenane.

**FIGURE 3: LEFT: FISH ASSAY AND RIGHT: CURRENT STRATEGY FOR SIGNAL ENHANCEMENT**




Left: FISH assay (red probe) on mitotic chromosomes of rye (*Secale cereale*) using two labels per FISH probe. Right: Current strategy for signal enhancement using dendritic structures.

## CONCLUSION

Other applications of the CuAAC that have been developed within the group of Prof. Carell in recent years include synthesis of labelled nucleotides,[7] and synthesis of probes for the sequencing of epigenetic markers.[8] Despite the numerous applications which have already been developed using CuAAC, many more are expected in the near future. Using new

ligands and applying improved reaction conditions, researchers have clicked on proteins,[9] on glycan structures and even *in vivo* in fish embryos.[10]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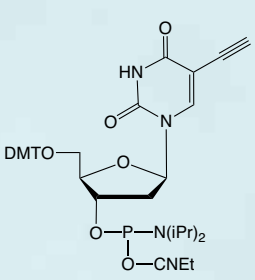
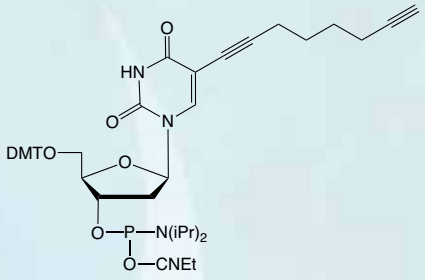
## NEW PRODUCT - TIPS-5-ETHYNYL-dU-CE PHOSPHORAMIDITE

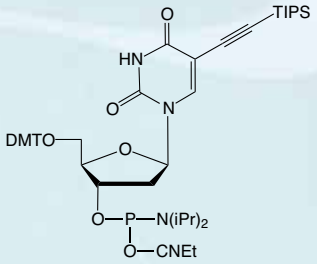
***FIGURE 1:*** **5-ETHYNYL-dU, ITS TIPS PROTECTED ANALOGUE AND C8-ALKYNE-dT**




*(1) 5-Ethynyl-dU-CE Phosphoramidite*




*(2) C8-Alkyne-dT-CE Phosphoramidite*




*(3) TIPS-5-Ethynyl-dU-CE Phosphoramidite*

First introduced in 2002, Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) is a robust and versatile Click Chemistry methodology commonly characterized by high yields, simple conditions, mild reagents, innocuous by-products, and simple purification.[1-3] Subsequent developments by Carell and Seela with copper-stabilizing ligands that protect DNA from copper-induced damage and alkyne-bearing modifications for oligonucleotide synthesis have produced a versatile method that is fully compatible with oligonucleotide labelling.[4-8]

In 2010, Glen Research partnered with baseclick GmbH to offer alkyne-bearing phosphoramidites, azides, and ancillary reagents for labelling oligonucleotides using CuAAC Click conjugations. Through an updated agreement with baseclick we are pleased to continue to offer and expand our list of these versatile reagents for labelling of oligonucleotides for research and development use.

Previous work incorporating 5-Ethynyl-dU-CE Phosphoramidite (1) in oligonucleotide synthesis showed that this modified nucleoside worked well, albeit with some restrictions. Unlike the C8-Alkyne-dT-CE Phosphoramidite (2), 5-ethynyl-dU is subject to base-catalyzed hydration during cleavage and deprotection, especially when using a strong base or heat.[9] Hydration of an ethynyl group forms a methyl ketone which subsequently blocks potential click reactions. Mild deprotection conditions are necessary to prevent this side reaction. Acid catalyzed reactions are also possible for 5-ethynyl-dU although we did not observe any degradation in our short test oligos.

In this article, we introduce TIPS-5-Ethynyl-dU-CE Phosphoramidite (3). This protected alkyne offers broader compatibility with oligonucleotide synthesis and deprotection. Protecting the 5-ethynyl group with a triisopropylsilyl (TIPS) protecting group prevented acid or base catalyzed hydration during oligonucleotide synthesis and workup.[9] A quick treatment with TBAF removed the TIPS protecting group. A simple ethanol precipitation or Glen Gel-Pak™ desalting step produced very pure oligos suitable for CuAAC click conjugations.

## PRODUCT INFORMATION

TIPS-5-Ethynyl-dU Phosphoramidite requires a 3 minute coupling time. Oligonucleotide cleavage and deprotection can be carried out according to the requirements of the nucleobases. After deprotection, the oligonucleotide is dried. A variety of protocols are then available for removing the TIPS protecting group but we found the following simple protocol worked very well.

*Protocol for TIPS removal*

1. Dissolve the oligonucleotide in 0.4mL anhydrous DMF.
2. Transfer the solution to a plastic centrifuge tube.
3. Add 0.1mL tetrabutylammonium fluoride (TBAF) to the tube.
4. Deprotect at 45°C for 15 minutes.
5. Quench the reaction with 0.5mL 2M triethylammonium acetate (TEAA).
6. Desalt the oligonucleotide on a Glen Gel-Pak cartridge, or equivalent, and filter the product solution if necessary.

## PRODUCT HIGHLIGHTS

*5-Ethynyl-dU Phosphoramidite compatibility*

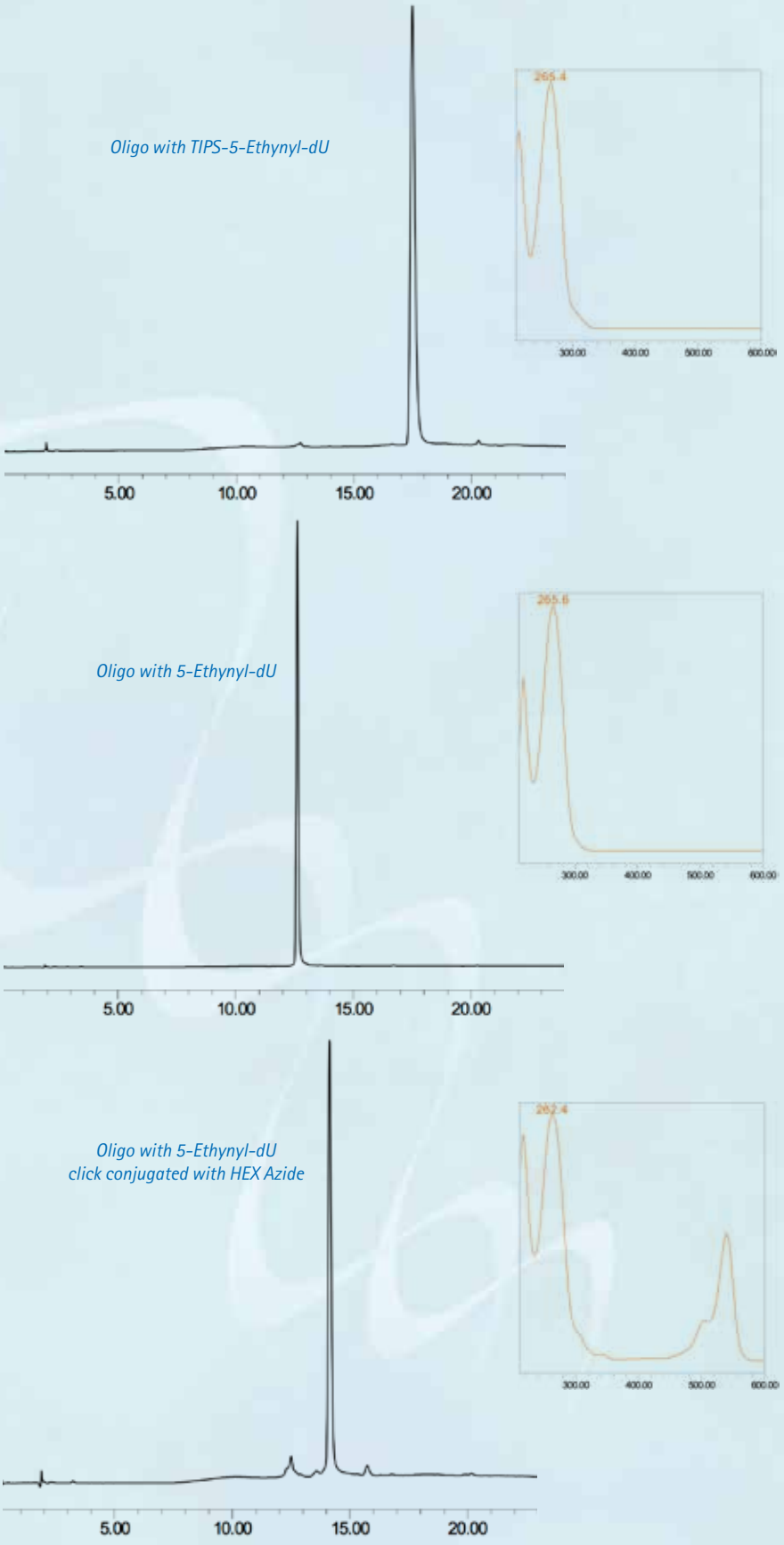
- ✔ ammonium hydroxide deprotection at room temperature
- ✗ not compatible with ammonium hydroxide AND heat
- ✔ potassium carbonate deprotection
- ✗ not compatible with any AMA deprotection

*TIPS-5-Ethynyl-dU Phosphoramidite compatibility*

- ✔ ammonium hydroxide at 55°C for 17 hours
- ✔ ammonium hydroxide deprotection at room temperature
- ✔ potassium carbonate deprotection
- ✔ AMA deprotection at 65°C for 10 minutes
- ✔ AMA deprotection at room temperature for 2 hours

*(Continued on Back Page)*

***FIGURE 2:*** **RP HPLC ANALYSIS OF 12-MER OLIGO BEFORE AND AFTER CLICK CONJUGATION WITH HEX**



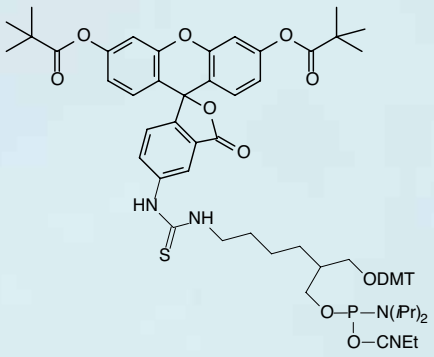
Fluorescein may not be the ideal label for oligonucleotides due to the pH dependence of its fluorescence (fluorescent only at pH >7) and its tendency to photobleach. Regardless, fluorescein remains one of the most popular labels for oligonucleotides. Over many years, Glen Research has accumulated a wide variety of products for fluorescein labelling and, in this article, we will describe the pros and cons of our various options for 3' and 5' labelling.

In the early days, the most popular reagent for fluorescein labelling in general was fluorescein isothiocyanate (FITC), so it was natural for us to offer products for 'FITC labelling' optimized for oligonucleotide synthesis. Our first products were the FITC derived phosphoramidite (1) and CPG (2), based on the 1,3-diol backbone developed by Nelson.[1] Both of these products remain popular and allow very efficient fluorescein labelling. However, as mass spectrometric analysis became more popular, it became clear that there was an inherent flaw in these products. The thiourea linkage derived from FITC is not stable[2] to deprotection conditions and is susceptible to side reactions, including hydrolysis to a urea linkage and ammonolysis to a guanidine linkage, as shown in Figure 2. While these side reactions have no effect on the fluorescein content, they do complicate the MS analysis and potentially RP HPLC analysis of singly and especially doubly labelled oligonucleotides.

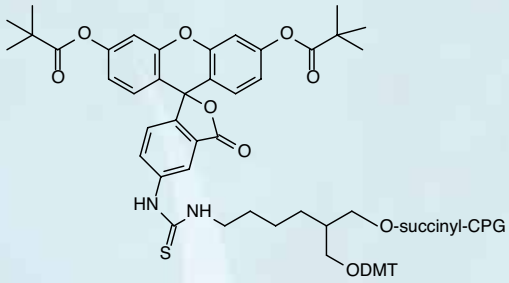
Soon thereafter, 5'-fluorescein phosphoramidite (6-FAM) (3) was introduced and this product was prepared using 6-carboxyfluorescein as a single isomer. 6-FAM rapidly became the most popular fluorescein labelling product since it simply generates a pure oligonucleotide labelled at the 5' terminus. So, it was natural for us to apply the 6-FAM approach to our branched product line and we introduced 6-fluorescein phosphoramidite (5) and the equivalent 3'-(6-fluorescein) CPG (6). These two products offer the benefits of 6-FAM (simplicity, isomeric purity and minimal side reactions) along with the ability to purify derived oligos by Glen-Pak™ DMT-ON purification.

Many years ago, we also introduced 3'-(6-FAM) CPG (4), which is based on the

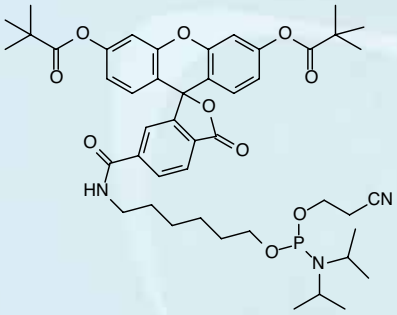
***FIGURE 1:*** **STRUCTURES OF FLUORESCEIN PHOSPHORAMIDITES AND SUPPORTS**



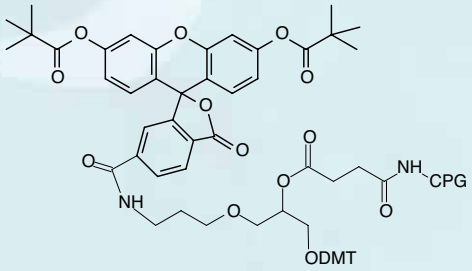

*(1) Fluorescein Phosphoramidite (10-1963)*



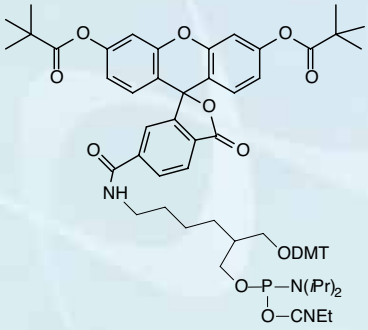

*(2) 3'-Fluorescein CPG (20-2963)*



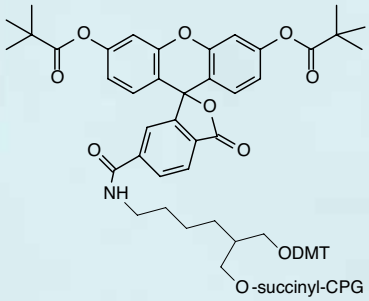

*(3) 5'-Fluorescein Phosphoramidite (10-5901)*



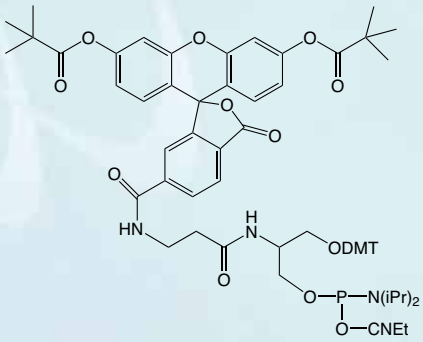

*(4) 3'-(6-FAM) CPG (20-2961)*



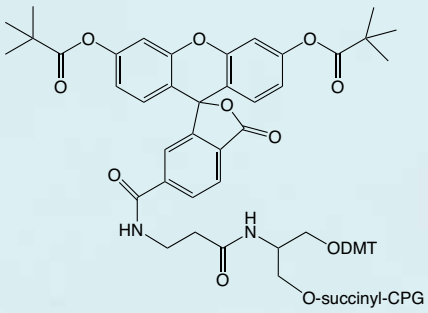

*(5) 6-Fluorescein Phosphoramidite (10-1964)*




*(6) 3'-(6-Fluorescein) CPG (20-2964)*




*(7) 6-Fluorescein Serinol Phosphoramidite (10-1994)*




*(8) 3'-6-Fluorescein Serinol CPG (20-2994)*

well used 1,2-diol backbone. We still offer this product and its popularity has never waned. However, as we have demonstrated in past newsletters, this type of backbone is susceptible to an elimination reaction during deprotection that leads to loss of label.

More recently, we introduced 6-fluorescein serinol phosphoramidite (7) and the equivalent 3'-6-fluorescein serinol CPG (8) based on our increasingly popular serinol-based 1,3-diol linkage.[3] These products offer equivalent performance levels to phosphoramidite (4) and CPG (5).

## EXPERIMENTAL DESIGN

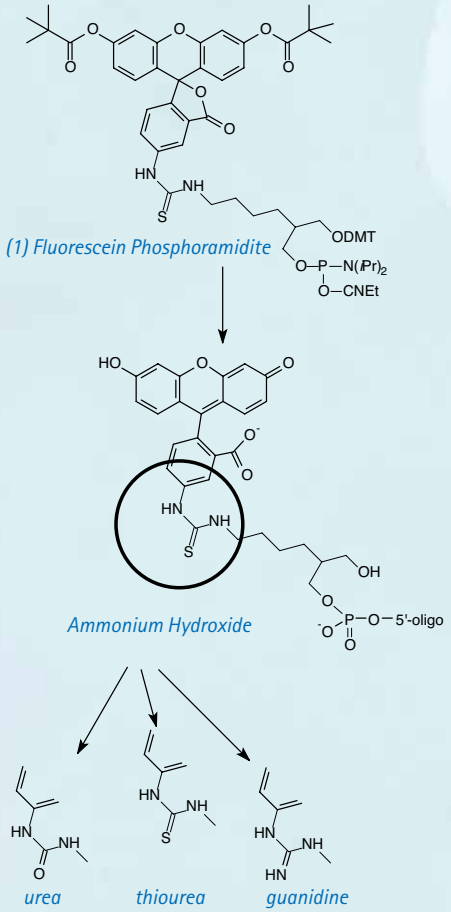
In order to take a close look at the performance of these eight fluorescein products, we have carried out a simple set of experiments designed to illustrate the strength and weakness of each product.

In the case of the phosphoramidites, each was added to the 5' terminus of a simple T6 oligo. Where apropriate, the final DMT group was removed and the crude oligo was analyzed by reverse phase HPLC.

In the case of each support, a T6 oligonucleotide was synthesized followed by deprotection and DMT-ON purification using Glen-Pak cartridges. The DMT-ON purification was designed to remove any early failures so that we could focus entirely on the 3' linkage to fluorescein.

In all cases, the oligonucleotides were deprotected using ammonium hydroxide at 55 °C for 17 hours to closely mimic the deprotection of regular oligonucleotides.

**FIGURE 2: HYDROLYSIS OF C=S**




## RESULTS

### 5' Terminus

As expected, the 5'-fluorescein phosphoramidite (3) performed impeccably, yielding a product oligonucleotide, which was essentially 100% pure. The results for 6-Fluorescein, as shown in Figure 3a, and 6-Fluorescein Serinol Phosphoramidites were the same at close to 100% purity. However, the results for Fluorescein Phosphoramidite looked even worse than we had expected. The chromatogram showed several impurity

## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| 5'-Fluorescein Phosphoramidite (6-FAM) | 10-5901-95 | 50 µmole | 110.00 |
| | 10-5901-90 | 100 µmole | 215.00 |
| | 10-5901-02 | 0.25g | 575.00 |
| Fluorescein Phosphoramidite | 10-1963-95 | 50 µmole | 165.00 |
| | 10-1963-90 | 100 µmole | 295.00 |
| | 10-1963-02 | 0.25g | 595.00 |
| 6-Fluorescein Phosphoramidite | 10-1964-95 | 50 µmole | 165.00 |
| | 10-1964-90 | 100 µmole | 295.00 |
| | 10-1964-02 | 0.25g | 595.00 |
| 6-Fluorescein Serinol Phosphoramidite | 10-1994-95 | 50 µmole | 165.00 |
| | 10-1994-90 | 100 µmole | 295.00 |
| | 10-1994-02 | 0.25g | 595.00 |
| 3'-Fluorescein CPG | 20-2963-01 | 0.1g | 120.00 |
| | 20-2963-10 | 1.0g | 995.00 |
| 1 µmole columns | 20-2963-41 | Pack of 4 | 200.00 |
| 0.2 µmole columns | 20-2963-42 | Pack of 4 | 120.00 |
| 10 µmole column (ABI) | 20-2963-13 | Pack of 1 | 300.00 |
| 15 µmole column (Expedite) | 20-2963-14 | Pack of 1 | 450.00 |
| 3'-(6-Fluorescein) CPG | 20-2964-01 | 0.1g | 120.00 |
| | 20-2964-10 | 1.0g | 995.00 |
| 1 µmole columns | 20-2964-41 | Pack of 4 | 200.00 |
| 0.2 µmole columns | 20-2964-42 | Pack of 4 | 120.00 |
| 10 µmole column (ABI) | 20-2964-13 | Pack of 1 | 300.00 |
| 15 µmole column (Expedite) | 20-2964-14 | Pack of 1 | 450.00 |
| 3'-(6-FAM) CPG | 20-2961-01 | 0.1g | 120.00 |
| | 20-2961-10 | 1.0g | 995.00 |
| 1 µmole columns | 20-2961-41 | Pack of 4 | 200.00 |
| 0.2 µmole columns | 20-2961-42 | Pack of 4 | 120.00 |
| 10 µmole column (ABI) | 20-2961-13 | Pack of 1 | 300.00 |
| 15 µmole column (Expedite) | 20-2961-14 | Pack of 1 | 450.00 |
| 3'-6-Fluorescein Serinol CPG | 20-2994-01 | 0.1g | 120.00 |
| | 20-2994-10 | 1.0g | 995.00 |
| 0.2 µmole columns | 20-2994-42 | Pack of 4 | 120.00 |
| 1 µmole columns | 20-2994-41 | Pack of 4 | 200.00 |
| 10 µmole column (ABI) | 20-2994-13 | Pack of 1 | 300.00 |
| 15 µmole column (Expedite) | 20-2994-14 | Pack of 1 | 450.00 |

peaks but the UV/Visible spectrum of all components confirmed the presence of fluorescein, as shown in Figure 3b.

*3'-Terminus*

Oligonucleotide synthesis with the label at the 3' terminus is much more difficult than at the 5' terminus. The label itself is challenged by all of the reagents of oligonucleotide synthesis in multiple cycles. Similarly, the linker is susceptible to side reactions.

In our experiments, three of the supports, 3'-(6-Fluorescein), 3'-(6-FAM) and 3'-6-Fluorescein Serinol performed very well with only minor side reactions being observed at the linker. One surprise for us was that 3'-(6-FAM), attached by a 1,2-diol based linker and perfectly set up for an elimination reaction, did not lead to any appreciable loss of label in this experiment, as shown in Figure 3c.

On the other hand, 3'-Fluorescein exhibited even more side reactions at the thiourea linker than expected, as shown in Figure 3d, but, again, all peaks contained fluorescein. In addition to the expected hydrolysis and ammonolysis side products illustrated in Figure 2, mass spectroscopic analysis showed there were also components in the mixture with additional sulfur in the oligos. This may indicate that the hydrolytic mechanisms leading to loss of sulfur can also cause sulfurization of adjacent phosphodiester linkages to yield a family of phosphorothioate modifications.

## CONCLUSION

All of our fluorescein products performed well in these tests. In particular, 6-Fluorescein phosphoramidite (10-1964) and CPG (20-2964) and 6-Fluorescein Serinol (10-1994) and CPG (20-2994) proved to be very strong performers as phosphoramidites and supports, with minimal side reactions related to their 1,3-diol based linkers.

One surprise was the very high quality of product derived from 3'-(6-FAM) CPG (20-2961). In this experiment, we did not observe any significant elimination of the 3' linkage with attendant loss of fluorescein.

**FIGURE 3: RP HPLC OF FLUORESCEIN CONTAINING OLIGOS**

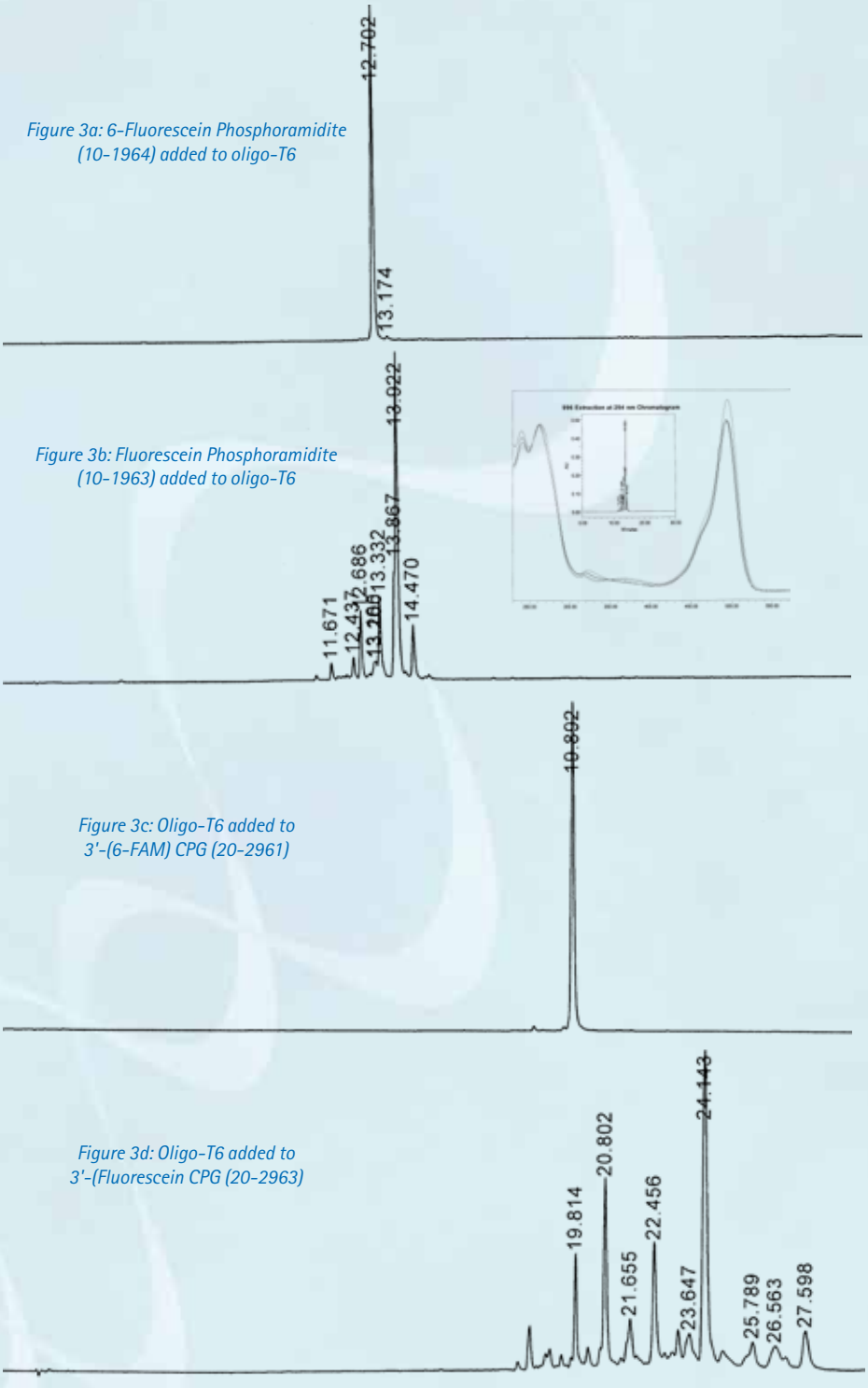



*Figure 3a: 6-Fluorescein Phosphoramidite (10-1964) added to oligo-T6*

*Figure 3b: Fluorescein Phosphoramidite (10-1963) added to oligo-T6*

*Figure 3c: Oligo-T6 added to 3'-(6-FAM) CPG (20-2961)*

*Figure 3d: Oligo-T6 added to 3'-(Fluorescein CPG (20-2963)*

However, it is clear that the thiourea linker derived from FITC in Fluorescein Phosphoramidite (10-1963) and CPG (20-2963) should not be used for any more complex experiments than simple 5' or 3' labelling.

*References:*


1. P.S. Nelson, M. Kent, and S. Muthini, *Nucleic Acids Research,* 1992, **20**, 6253-6259.
2. I. Dubey, G. Pratviel, and B. Meunier, *Bioconjug Chem,* 1998, **9**, 627-32.
3. US Patent No.: 8,394,948 (http://www.glenresearch.com/GlenReports/GR25-14.html)

## NEW PRODUCTS – AQUAPHLUOR® 593 5′-PHOSPHORAMIDITE AND CPG

Glen Research has been active in supplying fluorescent dyes for labelling oligonucleotides for close to 30 years. Indeed, fluorescent dyes are probably the most commonly used tags for modifying oligonucleotides since they offer such sensitive detection in a wide variety of techniques varying from sequencing to genetic analysis. We offer a broad range of fluorophores covering most of the fluorescence spectrum. However we have never had a good substitute for Texas Red® or Alexa Fluor® 594, both of which are not amenable to the conditions of oligonucleotide synthesis and deprotection.

To be a viable alternative to Texas Red®, a fluorophore should exhibit the following desirable attributes for oligonucleotide synthesis:

- Its absorbance maximum should be in the range 590 - 600nm with a fluorescence maximum around 615nm.
- It should be stable to standard deprotection conditions.
- Its brightness should be insensitive to changes in pH.
- Its emission - unlike other dyes, e.g., cyanine dyes - should not be significantly quenched with increasing temperature.

Our long-term relationship with the Epoch Biosciences division of ELITechGroup has allowed us to evaluate several of their proprietary AquaPhluor® family of fluorescent dyes. AquaPhluor dyes have been developed to allow access to fluorophores containing large fluorescent aromatic heterocycles with their natural hydrophobicity substantially modulated by the presence of a charged phosphonate group in the molecule. For phosphoramidite chemistry, the latent phosphonate group is elegantly protected with a trifluoroacetamidobutyl (TFAAB) group, which is removed during base deprotection by a mechanism shown in Figure 1. The resulting negatively charged phosphate forms a hydrophilic zwitterion with the positively charged dye.

AquaPhluor® 593 (AP593) (1) has the properties which led us to believe that this fluorophore could be a good substitute for Texas Red®. It is a highly fluorescent molecule with an absorption maximum at 593nm and an emission maximum at 613nm. Its quantum yield is 0.6. The fluorescence of AquaPhluor® 593 is only weakly affected by pH, varying only a few percent in the range pH 5-8. In addition, the fluorescence of AquaPhluor® 593 only drops about 30% when heated from 20 °C to 95 °C.

We are delighted to offer 5′-AquaPhluor® 593 Phosphoramidite (2) and 3′-AquaPhluor® 593 CPG (3), the structures of which are shown in Figure 2.

***FIGURE 1:*** **MECHANISM OF REMOVAL OF TFAAB PROTECTING GROUP**

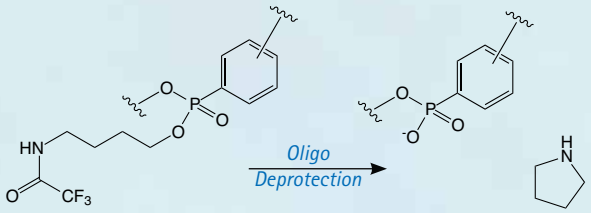
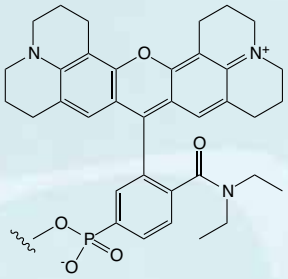
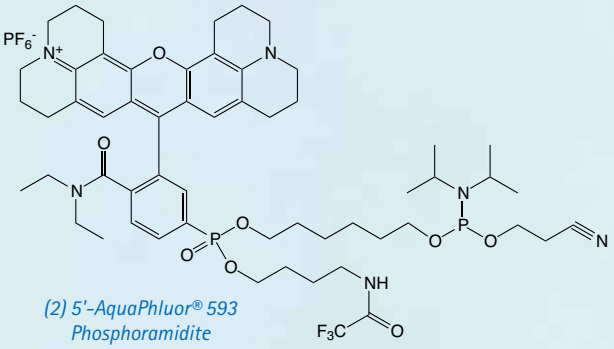


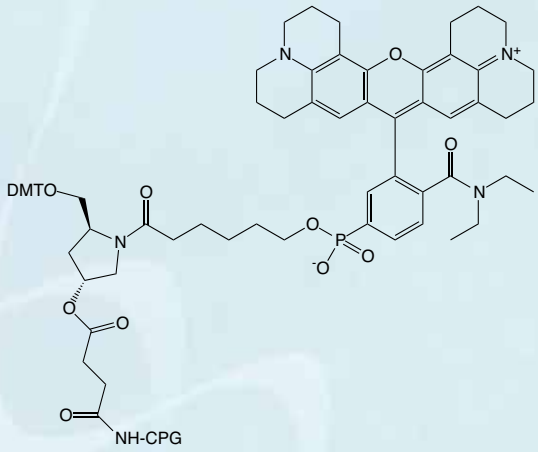

***FIGURE 2:*** **AQUAPHLUOR® 593, 5'-PHOSPHORAMIDITE AND 3'-CPG**



*(1) AquaPhluor® 593 (AP593)*




*(2) 5′-AquaPhluor® 593 Phosphoramidite*




*(3) AquaPhluor® 593 CPG*

### *5′-AquaPhluor® 593 Phosphoramidite*

A 3 minute coupling time is recommended.

### *AquaPhluor® 593 CPG*

This support should be used in a manner identical to a normal protected nucleoside support since it contains the DMT group.

### *AquaPhluor® Deprotection with Ammonium Hydroxide*

Oligonucleotides containing AquaPhluor® 593 can be deprotected using ammonium hydroxide with a few provisos. The following conditions gave excellent results:

- 2 hr at 65 °C
- 17 hr at room temperature
- 17 hr at 55°C

However, the last more aggressive

treatment led to a small amount of degradation of the dye.

It should be noted that an interesting side reaction of AquaPhluor® 593 was observed when using a fast-cleaving support, e.g., Glen UnySupport FC or a Q support. This side reaction leads to a non-fluorescent impurity. However, this impurity, formed only during deprotection with ammonium hydroxide using a fast cleaving support, can be eliminated using a pre-treatment with 10% diethylamine in acetonitrile for 2-3 minutes (Figure 3a). More details are available in this link: http://www.glenresearch.com/GlenReports/GR28-2R.html

### *AquaPhluor® Deprotection with AMA*

Oligonucleotides containing AquaPhluor® 593 can be deprotected using AMA and no pre-treatment with a hindered base is required, as shown in Figure 3b.

- 10 minutes at 65 °C

### *Purification*

5'-AquaPhluor® 593 is not sufficiently hydrophobic to be compatible with standard Glen-Pak purification protocols. However, a suitable, modified Glen-Pak™ purification protocol is being investigated. Please inquire.

### *Conclusion*

We have demonstrated that AquaPhluor® 593 is a dye that is stable to the conditions of oligonucleotide synthesis and deprotection. Its emission fits nicely in the range of emission between Cyanine 3.5 and Cyanine 5 dyes. AquaPhluor® 593 is relatively insensitive to changes in pH in the range pH 5-8. In addition, the emission of AquaPhluor® 593 is not substantially quenched by raising the temperature from 20 - 95 °C. We have concluded that AquaPhluor® 593 is a dye with superior chracteristics and is indeed a viable alternative to Texas Red® and Alexa Fluor® 594.

We are happy to provide 5'-AquaPhluor® 593 Phosphoramidite and 3'-AquaPhluor® 593 CPG in collaboration with ELITechGroup.

### *IP Statement*



**FIGURE 3: RP HPLC ANALYSIS OF 5'-AQUAPHLUOR® 593 LABELLED OLIGOS**

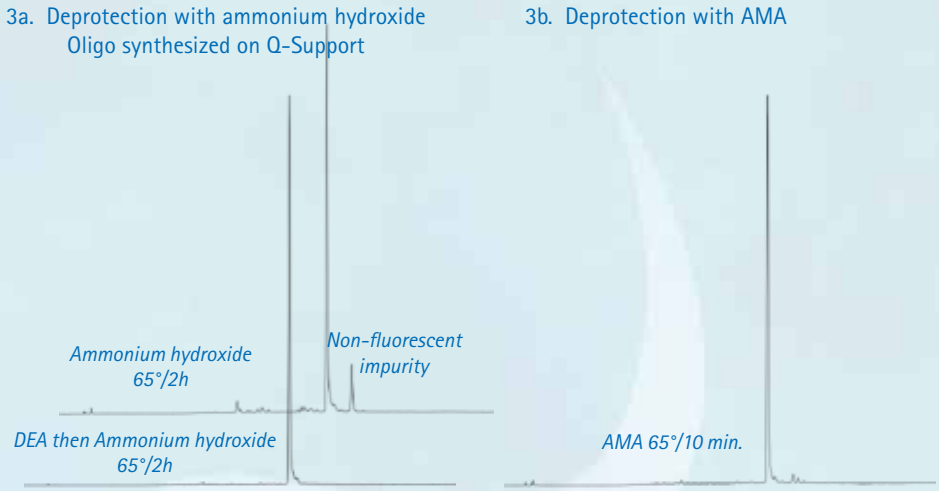
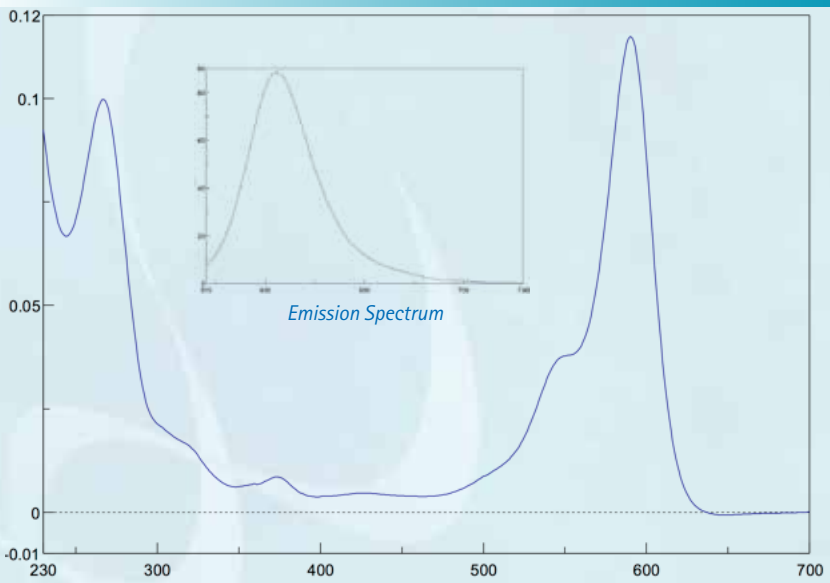



**FIGURE 4: ABSORBANCE AND EMISSION SPECTRA OF AQUAPHLUOR® 593**




*Absorbance Spectrum of 5'-AquaPhluor® 593 T6 in 0.1M TEAA, pH 7*

## *ORDERING INFORMATION*

| *Item* | *Catalog No.* | *Pack* | *Price($)* |
|---|---|---|---|
| 5'-AquaPhluor® 593 Phosphoramidite | 10-5923-95 | 50 µmole | 405.00 |
| | 10-5923-90 | 100 µmole | 795.00 |
| | 10-5923-02 | 0.25g | 1575.00 |
| AquaPhluor® 593 CPG | 20-5923-01 | 0.1g | 215.00 |
| | 20-5923-10 | 1.0g | 1800.00 |
| 1 µmole columns | 20-5923-41 | Pack of 4 | 325.00 |
| 0.2 µmole columns | 20-5923-42 | Pack of 4 | 165.00 |
| 10 µmole column (ABI) | 20-5923-13 | Pack of 1 | 925.00 |
| 15 µmole column (Expedite) | 20-5923-14 | Pack of 1 | 1395.00 |

## NEW PRODUCT: GLEN-PAK™ DNA 3mg 384-WELL PLATE

The Glen-Pak™ product line continues to be one of the industry's most flexible options for fast, high-fidelity purification of DMT-ON oligonucleotides. While the Glen-Pak DNA cartridge for use with disposable syringes (60-5200-xx) is still the go-to option for initial methods development, many of our customers in need of higher throughput are using our three 96-well products to purify their DMT-ON oligos.

The standard DNA cartridge (60-5100-xx) is used to purify at synthesis scales of up to 1.0 µmole and our 50mg version, contained in a tube of the same dimensions (60-5000-96), allows single-step reagent additions at the 200 nmole scale. Finally, the Glen-Pak 30mg 96-well plate gives customers the option to purify up to 50 nmole scale syntheses in a standardized, deep well format. All three of these products allow customers to purify up to 96 oligos in one run using off-the-shelf vacuum manifolds while the standard and 50mg versions are also compatible with chamber/port based solid-phase extraction (SPE) systems.

Examples of our early Glen-Pak options are shown in the top right photo.

### NEW TECHNOLOGY SETTING THE PACE

The recent introduction of DNA/RNA synthesizers using support-impregnated, 384-well filter plates has opened up even higher throughput production options for those desiring higher synthesis throughput. As was the case when 96-well systems first hit the market, customers have requested a way to seamlessly move from whole-plate cleavage and deprotection to DMT-ON, Glen-Pak purification. In response to this new demand, we are happy to introduce our highest throughput purification product yet, the Glen-Pak DNA 3mg 384-Well Plate shown in the lower right photo.

The Glen-Pak DNA 3mg 384-Well Plate is designed for use with 384-well plate compatible vacuum manifold systems and can purify up to a 20 nmole scale synthesis. Each well contains 3mg of Glen-Pak DNA resin, which binds about 15 nmoles of full length 40-mer DMT-ON oligo.

*GLEN-PAK™ EARLY OPTIONS*

*GLEN-PAK™ DNA 3mg 384-WELL PLATE*


The low resin component in each well opens up the opportunity to normalize final product yields across the plate at the purification stage. A simple over load of crude, DMT-ON product, capturing the maximum amount of full-length material, allows higher fidelity downstream processing and quantification. Such a step decreases the impact of crude synthesis quality on final yield variation across the plate and allows quick identification of well-to-well anomalies. Of course, loading less than 20 nmoles in order to capture all DMT-ON material from a crude synthesis for purification is still a fine strategy.

As with any high-throughput system, customers will have to develop their own liquid-handling methods based on the specific equipment being used in downstream processing. That said, we have found the Glen-Pak DNA protocol to be easily scaled based on resin content and a simple reduction in volumes from our standard Glen-Pak DNA cartridge protocol:

http://www.glenresearch.com/Technical/GlenPak_UserGuide.pdf

## BENEFITS OF THE 384-WELL FORMAT

- Potential for normalization of yield, which aids in downstream processing.
- Lower cost processing (less labor and unattended operation if using a robot).
- Less processing time per oligo when using a whole plate.
- Less solvent usage per oligonucleotide for every purification step.
- Less final elution volumes to dry in preparation for analysis or use.

## BENEFITS OF GLEN-PAK OVER STANDARD SPE PURIFICATION METHODS

Glen-Pak cartridges are highly versatile due to their ability to be used directly with common base deprotection solutions such as Ammonium Hydroxide, AMA, Tert-Butylamine/Water, 0.4M NaOH Methanol/Water 4:1, and Potassium Carbonate in Methanol.

They impart a larger capacity per gram of bed volume than most standard reverse phase cartridge systems due to the highly specific capture of DMT-ON products and simultaneous exclusion of DMT-Off failure sequences during the loading step.

Glen-Pak products are compatible with oligonucleotides over a very broad length range. We have successfully purified DNA sequences from 2 to 150 bases in length and RNA sequences over 60 bases long.

Finally, there are multiple scales and formats available to meet downstream processing requirements ranging from single use to multi-well automation, since RNA and DNA Glen-Paks can be used with a standard, disposable syringe in addition to multi-well and chamber/port vacuum manifolds.

Please see the Glen-Pak User Guide for specific protocols:

http://www.glenresearch.com/Technical/GlenPak_UserGuide.pdf



**TABLE: GLEN-PAK™ PRODUCT LINE SUMMARY**

| Description | Catalog No. | Scale | Format | Uses |
|---|---|---|---|---|
| Glen-Pak DNA 3mg | 60-5500-xx | Up to 20 nmole | 384 Well Plate | High throughput<br>Routine DNA |
| Glen-Pak DNA 30mg | 60-5400-01 | Up to 50 nmole | 96 Well Plate | High throughput<br>Routine DNA |
| Glen-Pak DNA 50mg | 60-5000-96 | Up to 200 nmole | Cartridge | High throughput<br>Routine DNA<br>Low volumes used |
| Glen-Pak DNA | 60-5100-xx | Up to 1 µmole | Cartridge | High throughput<br>Routine DNA<br>Up to 150mers |
| Glen-Pak DNA | 60-5200-xx | Up to 1 µmole | Hand Held Cartridge | Routine DNA |
| Glen-Pak RNA | 60-6100-xx | Up to 1 µmole | Cartridge | High throughput<br>Routine RNA |
| Glen-Pak RNA | 60-6200-xx | Up to 1 µmole | Hand Held Cartridge | Routine RNA |
| Glen-Pak DNA 3g | 60-5300-01 | Up to 20 µmole | Cartridge | Routine DNA |

## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| *DNA Purification Cartridges* | | | |
| Glen-Pak™ 50mg DNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices) | 60-5000-96 | Pack of 96 | 415.00 |
| Glen-Pak™ DNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices) | 60-5100-10 | Pack of 10 | 80.00 |
| | 60-5100-30 | Pack of 30 | 200.00 |
| | 60-5100-96 | Pack of 96 | 475.00 |
| Glen-Pak™ DNA Purification Cartridge (For use with disposable syringes) | 60-5200-01 | each | 8.00 |
| | 60-5200-10 | Pack of 10 | 80.00 |
| Glen-Pak™ DNA Cartridge 3g | 60-5300-01 | Pack of 1 | 150.00 |
| Glen-Pak™ DNA 30mg 96-Well Plate | 60-5400-01 | Pack of 1 | 475.00 |
| Glen-Pak™ DNA 3mg 384-Well Plate | 60-5500-01 | Pack of 1 | 675.00 |
| | 60-5500-10 | Pack of 10 | 6750.00 |
| *RNA Purification Cartridges* | | | |
| Glen-Pak™ RNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices) | 60-6100-10 | Pack of 10 | 95.00 |
| | 60-6100-30 | Pack of 30 | 225.00 |
| | 60-6100-96 | Pack of 96 | 575.00 |
| Glen-Pak™ RNA Purification Cartridge (For use with disposable syringes) | 60-6200-01 | each | 9.50 |
| | 60-6200-10 | Pack of 10 | 95.00 |

GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164



*(Continued from Page 4)*

Previous Glen Reports have highlighted strategies and applications for Click conjugation.[10] Some of the diverse strategies include solid and aqueous-phase conjugations, small and large-scale conjugations, and multiple click reporters. Applications include crosslinking, ligation, spin labels, and photo-crosslinking. We offer a diverse set of reagents for modifying oligonucleotides, including terminal modifiers, internal nucleosidic modifiers, non-nucleosidic modifiers, and post synthesis modifiers.

*References:*

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9. S.A. Ingale, H. Mei, P. Leonard, and F. Seela, *The Journal of Organic Chemistry*, 2013, **78**, 11271-82.
10. The Glen Report, 2014, **26.1**, 7-8. http://www.glenresearch.com/GlenReports/GR26-15.html

## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| 5-Ethynyl-dU-CE Phosphoramidite | 10-1554-95 | 50 µmole | 130.00 |
| | 10-1554-90 | 100 µmole | 245.00 |
| | 10-1554-02 | 0.25g | 775.00 |
| TIPS-5-Ethynyl-dU-CE Phosphoramidite | 10-1555-95 | 50 µmole | 195.00 |
| | 10-1555-90 | 100 µmole | 370.00 |
| | 10-1555-02 | 0.25g | 975.00 |
| C8-Alkyne-dT-CE Phosphoramidite | 10-1540-95 | 50 µmole | 165.00 |
| | 10-1540-90 | 100 µmole | 315.00 |
| | 10-1540-02 | 0.25g | 900.00 |