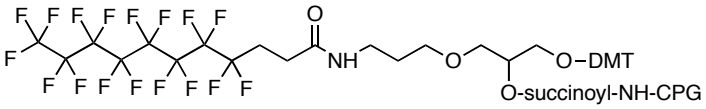
3'-Fluorous Modifier CPG
Product No. FL 1610
Product Information



Installs a permanent fluorous tag at the 3'-terminus of oligonucleotide. May be used for fluorous affinity purification and/or imparting fluorophilic or hydrophobic properties to an oligonucleotide.

Introduction: Fluorous Affinity Interactions. Highly fluorinated organic compounds are both hydrophobic and lipophobic, preferring instead to associate with other fluorinated substances. Organic molecules that have both an organic domain and a perfluoroalkyl domain (e.g., a linear perfluoroalkyl "ponytail") are known as *fluorous molecules*[1] (not to be confused with *fluorescent* molecules!), and may be separated from non-fluorous molecules by interaction with fluorinated separation media such as Fluoro-Pak columns.[2] Fluorous-fluorous interactions are strong and selective ("like dissolves like"). Early work in the oligonucleotide field focused on the use of fluorous interactions for purifications.[2] Specifically, Berry & Associates introduced the Fluorous Affinity Purification of Oligonucleotides, a higher affinity alternative to DMT-on reversed-phase cartridge purification. It relies on the strong interaction of fluorous-tagged oligonucleotides with the fluorous adsorbent in Fluoro-Pak columns. The fluorous tag took the form of a fluorous dimethoxytrityl (FDMT) group, which was installed using the appropriate FDMT-bearing nucleoside phosphoramidite. After fluorous purification on Fluoro-Pak columns with on-column detritylation, high recoveries of oligonucleotides were obtained, free from failure sequences, even with 100-mers.[2] The FDMT group also facilitates RP-HPLC purification. *The current product offering deviates from this approach and installs a **permanent** fluorous tag.*

Fluorous Modifier CPG. While many of Berry & Associates' fluorous products focus on the purification of oligonucleotides, fluorous tags have other potential applications in nucleic acid chemistry. 3'-Fluorous Modifier CPG (FL 1610) is useful for placing a *permanent* fluorous tag at the 3'-terminus of an oligonucleotide. In addition to providing a purification handle, fluorous modifications enable applications where fluorophilicity or high hydrophobicity are desired. For example, the presence of a fluorous tag in an oligonucleotide may allow its immobilization onto fluorous-coated glass slides.[3,4] Alternatively, placing fluorous monomers at strategic sites in an oligonucleotide may allow intra- or intermolecular fluorous-fluorous interactions, enhancing the attraction between various regions of an oligonucleotide.[5]

Coupling, cleavage, and deprotection: 3'-Fluorous Modifier CPG is provided on 1000 Å controlled-pore glass and couples with high efficiency under the standard conditions recommended for popular synthesizers. The oligonucleotide may be prepared in trityl-on or trityl-off mode as desired; see below for HPLC considerations.

Cleavage from the support and nucleobase deprotection can be accomplished using standard techniques; the fluorous tag is very robust to deprotection conditions.

HPLC analysis: The fluorous-tagged oligonucleotide can be analyzed by RP-HPLC, but a modified elution profile is required in order to elute the strongly-retained tag-bearing peaks. For example, using a Waters Spherisorb ODS-2 C18 column (5 um, 150 x 4.6 mm), Mobile A = 0.1 M triethylammonium acetate (TEAA), Mobile B = MeCN, and a 1 mL/min flow rate, a gradient starting at 5-8% MeCN and rising to 80-90% MeCN is useful. The fluorous-tagged peak elutes at about 40-60% MeCN concentration. Note that since the fluorous tag is present at the 3'-terminus, nearly all of the oligonucleotides, including failure sequences, will be strongly retained and typically appear in a similar region of the chromatogram.

The presence of a DMT group at the 5'-terminus leads to a slight increase in retention time (ca. 1-2 min) as compared to an oligonucleotide with no DMT group. The main contributor to strong retention is the fluorous tag; hence, DMT-on and DMT-off materials appear in the same area of the chromatogram.

Fluoro-Pak Cartridge purification: Cartridge purification using a Fluoro-Pak Column (FP 7210 or FP 7220) and Loading Buffer (LB 7100) can be accomplished using a modification of the protocol found in "*User Guide: Fluorous Purification of Oligonucleotides*", which is included in with your purchase or may be downloaded at www.berryassoc.com/literature/fluorousguide.pdf. As usual, ammonia removal is not required before loading.

Changes to the protocol:

(1) Use 2-5% MeCN/0.1 M TEAA for the failure wash step (Step 4.6 in the User Guide) rather than 10% MeCN/0.1 M TEAA, or leave this step out entirely. There are very few oligonucleotides that do not have a fluorous tag, since nearly all chains originate from a fluorous-tagged 3'-monomer. Thus, failure sequences have a fluorous tag and cannot be separated from full-length material using cartridge purification.

(2) If the DMT group was removed prior to Fluoro-Pak purification, skip the on-column detritylation step (Step 4.7 in the User Guide).

(3) To elute the final oligonucleotide (Step 4.8 in the User Guide), use 1 mL of 50% aqueous MeCN instead of 20% aqueous MeCN.

References:

1. *Handbook of Fluorous Chemistry*; Gladysz, J. A.; Curran, D. P.; Horváth, I. T., Eds.; Wiley-VCH: Weinheim, **2004**.

2. Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K.-Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114-7122.

3. Fluorous Technologies, Inc., offers Fluorous Modified Glass Slides for the immobilization of fluorous-tagged molecules for microarray formation; see http://www.fluorous.com. The slides feature excellent spot morphology, high signal-to-noise ratios, low and uniform background fluorescence levels, and low non-specific binding, since the fluorous surface around the spot does not interact well with non-fluorous molecules. The ability to use the fluorous tag as both a purification handle and an immobilization handle is also an advantage. Further, the fluorous immobilization is potentially reversible.

4. (a) Pohl and co-workers detected carbohydrate-lectin interactions using fluorous modified slides bearing fluorous-tagged carbohydrates; see: Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162-13163. (b) Spring and co-workers showed that fluorous-tagged small molecules could be immobilized on fluorous modified a glass surface and used to facilitate detection of protein-ligand binding interactions; see: Nicholson, R. L.; Ladlow, M. L.; Spring, D. R. *Chem. Commun.* **2007**, 3906-3908. (c) Schreiber and co-workers employed fluorous-immobilized small-molecule arrays to screen for histone deacylase inhibitors; see: Vegas, A. J.; Bradner, J. E.; Tang, W.; McPherson, O. M.; Greenberg, E. F.; Koehler, A. N.; Schreiber, S. L. *Angew. Chem. Int. Ed.* **2007**, *46*, 7960-7964.

5. Examples: Placing fluorous tags in the stem region of molecular beacons or next to optical tags to enhance fluorescence quenching.