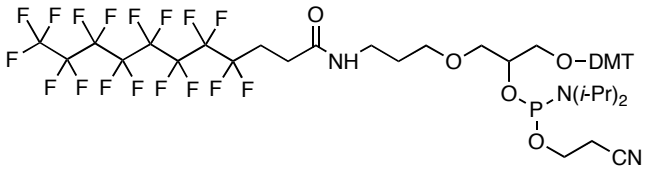
Fluorous Modifier CEP
Product No. FL 1600
Product Information



$C_{47}H_{53}F_{17}N_3O_7P$
Mol. Wt.: 1125.89

Installs a permanent fluorous tag internally or at the 5'-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 CEP. While many of Berry & Associates' fluorous products focus on the purification of oligonucleotides, fluorous tags have other potential applications in nucleic acid chemistry. Fluorous Modifier CEP (FL 1600) is useful for placing a *permanent* fluorous tag internally or at the 5'-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: Fluorous Modifier CEP couples with greater than 95% efficiency (typically >99%) under the standard conditions recommended for popular synthesizers. Extended coupling (e.g., 15 min) is not required, but leads to a slight (< 1%) improvement in coupling efficiency. Please note that while this reagent is freely soluble in acetonitrile, it is slow to dissolve. Allow ca. 15 minutes with occasional swirling for complete dissolution.

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

Caution: If Fluorous Modifier CEP is installed at the 5'-terminus, for best yields, *the oligonucleotide should be synthesized in trityl-on mode*. It is important that the terminal trityl group be present during cleavage/deprotection to avoid chain cleavage. After cleavage from the support and deprotection, the DMT group can be removed as usual (e.g., with 80% acetic acid).

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 peak. 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, the following gradient profile is useful: 5-30% B over 30 min, then 30-80% B over an additional 10 min. Failure sequences and DMT-bearing by-products at ca. 10-15 min and 15-20 min, respectively, followed by the fluorous-tagged peak at about 50-60% MeCN concentration. The presence or absence of a 5'-DMT group on the fluorous-tagged oligonucleotide does not change the retention time appreciably (ca. 1-2 min).

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.

(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.