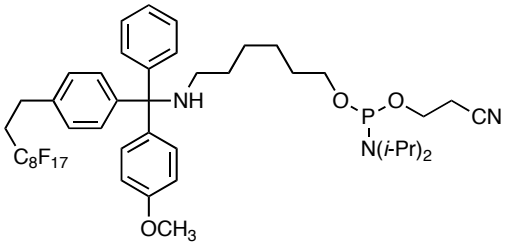
FMMT-5'-Amino-modifier-C6 CEP
Product No. FL 1500
Product Information



$C_{45}H_{51}F_{17}N_3O_3P$
Mol. Wt.: 1035.85

Installs an amine at the 5'-terminus of oligonucleotides with concomitant fluorous affinity purification.[1] *Employs the fluorous monomethoxytrityl (FMMT) group rather than an MMT group, providing greater discrimination of full-length oligonucleotides over failure sequences.*

For applications requiring the purification of 5'-amine-modified oligonucleotides, trityl-on purification is an option, typically involving a monomethoxytrityl (MMT) group. In cases where the separation of the trityl-on oligonucleotides from failure sequences and other non-trityl-bearing materials is more difficult, FMMT-5'-Amino-modifier-C6 CEP (FL 1500) is a useful option. It employs a fluorous version of the MMT group, i.e., a fluorous monomethoxytrityl (FMMT) group. This phosphoramidite behaves similarly to the MMT versions during synthesis, but the final FMMT-bearing oligonucleotide is more strongly retained on fluorous adsorbents or reversed-phase adsorbents, providing greater selectivity than observed for MMT-bearing oligos.

Coupling, cleavage, and deprotection: FMMT-5'-Amino-modifier-C6 CEP couples with greater than 95% efficiency (typically >98%) under the standard conditions recommended for popular synthesizers. Extended coupling is not required or recommended. Please note that while this reagent is freely soluble in acetonitrile, it is slow to dissolve. Allow 30 minutes with occasional swirling for complete dissolution, or use sonication if available.

Cleavage from the support can be accomplished using standard techniques. Nucleobase deprotection can be accomplished with concentrated ammonium hydroxide at 40 °C for 16 h.

HPLC analysis: The FMMT-tagged oligonucleotide can be analyzed by RP-HPLC, but a modified elution profile is required in order to elute the strongly-retained FMMT-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 FMMT-tagged peak at about 33-35 min (ca. 50-60% MeCN).

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.

Changes to the protocol:

(1) For best results, use more Fluoro-Pak adsorbent. For the purification of a 200 nmol synthesis, use an FP 7220 column (150 mg of adsorbent) rather than the normal FP 7210 column (75 mg of adsorbent). For larger syntheses, bulk adsorbent can be purchased from Berry & Associates, or call us for custom column sizes.

(2) Dilute the ammonia deprotection solution with 1.5-2x the volume of Loading Buffer (LB 7100).

(3) 5% MeCN/0.1 M TEAA should be used for the elution of the failure sequences rather than 10% MeCN/0.1 M TEAA.

(4) On-column detritylation should be avoided due to incomplete detritylation. Instead, elute the FMMT-bearing oligonucleotide with 1 mL of 50% aqueous MeCN. Further elution with 1 mL of 90% aqueous MeCN can be carried out as a safety measure for particularly strongly retained oligonucleotides. Remove the MeCN in vacuo, then detritylate with 80% acetic acid at room temperature for 1 h.

HPLC purification: Purify the FMMT-tagged oligonucleotide on an RP-HPLC column, then detritylate with 80% aqueous acetic acid. A standard RP-HPLC column (e.g., C18) may be employed in many cases, but for the highest resolution, a Fluoro-Pak HPLC column may be employed. Call for details.

The FMMT cation: FMMT cation λ_{max} = 477 nm in 0.1 M *p*-TsOH/MeCN. Compare to 472 nm for MMT cation.

References:

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