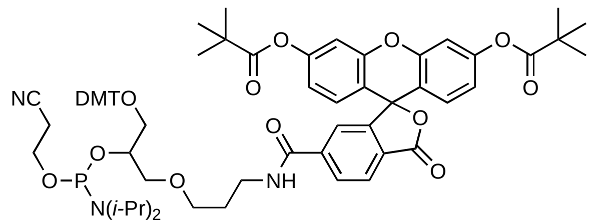
Fluorescein II CEP (6-FAM II, BA 0253)

Product Information



Installs a 6-carboxyfluorescein internally or at the 5'-terminus of an oligonucleotide using a DMT-bearing phosphoramidite, allowing DMT-on cartridge purification.

For the installation of fluorescein at the 5'-terminus of an oligonucleotide, the phosphoramidite "6-FAM" (5'-Fluorescein CEP, BA 0054), which does not bear a DMT group, is a popular choice. However, the lack of a trityl group precludes multiple additions or assaying the coupling step. We therefore offer Fluorescein II CEP ("6-FAM II", BA 0253), which features the same tether length as 6-FAM, but includes a DMT group.[1]

Coupling: Couple using normal instrument protocols except the coupling time should be extended to 15 min. Typical coupling yields are ca. 95%. Trityl-on mode is recommended (see below).

Cleavage and nucleobase deprotection: Use standard techniques. For the highest yields, prepare the oligo DMT-on and remove the trityl group after cleavage and deprotection. The DMT may also be used to facilitate cartridge purification with on-column detritylation, e.g. with Fluoro-Pak columns (see below).

Purification: Standard methods are applicable.

Fluoro-Pak™ columns may be used for cartridge purification with on-column detritylation if the final DMT group is left on. Use the following protocol:

1. Materials needed:

DMT-on oligonucleotide with 5'-fluorescein II installed. Ammonia may be present.
1 Fluoro-Pak™ or Fluoro-Pak™ II column (FP 7210 or FP 7220)
1 Luer adaptor (available with columns)
1 3 mL PE/PP syringe with male Luer tip
1 20 mL vial for catching waste
2 mL acetonitrile (MeCN)
3 mL 0.1 M aqueous TEAA (triethylammonium acetate)
2-5 mL Loading Buffer (Berry & Associates # LB-7100)
1 mL 3% aqueous TFA (trifluoroacetic acid)
1 mL 5% MeCN in 0.1 M aqueous TEAA
1 mL 50% MeCN in water

2. Sample preparation

Without removing the ammonia used in the deblocking step, dilute the crude deprotected oligonucleotide with an equal volume of Loading Buffer. The final sample volume should be 2-6 mL.

3. Conditioning the column

Pass the following through the Fluoro-Pak™ column to waste at a flow rate of 2 seconds per drop:

1. 2 mL of MeCN
2. 2 mL of 0.1 M aqueous TEAA
3. 2 mL of Loading Buffer

The first 2-3 drops of MeCN may be discolored.

4. Loading the DMT-on 6-FAM II-labeled oligonucleotide

Using the sample as prepared above, pass it through the pre-conditioned column at a flow rate of **5 seconds per drop**, conditions that allow one-pass loading. Most of the failure sequences pass through the column during loading.

5. Eluting remaining failure sequences

Pass the following through the Fluoro-Pak™ column to waste at a flow rate of **2 seconds per drop**:

1. 1 mL 5% MeCN in 0.1 M aqueous TEAA
2. 1 mL of water

The acetonitrile/TEAA solution selectively washes the remaining failure sequences from the resin while the fluorescein-labeled oligonucleotide is retained. The water step washes the buffer out of the resin prior to the acid-catalyzed detritylation in the next step.

6. On-column detritylation

Pass the following through the Fluoro-Pak™ column to waste at a flow rate of **2 seconds per drop**:

1. 2 mL 3% aqueous TFA. Column may become orange.
2. 1 mL of 0.1 M aqueous TEAA.
3. 1 mL of water.

7. Elution of the final detritylated oligonucleotide

Pass the following through the Fluoro-Pak™ column at **2 seconds per drop**, collecting the eluate in an appropriate sample tube:

1 mL of 50% acetonitrile in water. The oligo should elute in the first 15-20 drops.

Determine the optical density units at 494 nm to quantify the final oligonucleotide. Use as-is or lyophilize for short-term storage. If the oligonucleotide needs to be stored, add 100 mL of 10x TE buffer (100 mM Tris, 10 mM EDTA, pH 8) and store at 4 °C for a ready-use solution, or freeze at -20 °C for longer periods.

References

1. 5'-Fluorescein II CEP incorporates 6-carboxyfluorescein. The 5-carboxyfluorescein isomer of this product is also known and has been used with cartridge purification using OPC columns. See: Theisen, P.; McCollum, C.; Upadhya, K.; Jacobson, K.; Vu, H.; Andrus, A. *Tetrahedron Lett*. **1992**, *33*, 5033-5036.