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LightCycler® Fluorescein CPG

Version: 09
Content version: September 2016

For the synthesis of oligonucleotides labeled with fluorescein at the 3’ end

Cat. No. 03 138 178 001 1 g

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial/Bottle	Label	Function / Description	Content
1	LightCycler® Fluorescein CPG	▪ Contains LightCycler® Fluorescein long chain alkylamino Controlled Pore Glass (lcaa CPG) 500 Å for labeling of oligonucleotides at the 3' end. ▪ The reagent is supplied as a white solid. The loading of the CPG material is printed on the label.	1 bottle, 1 g

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C in a tightly sealed bottle, the reagent is stable through the expiration date printed on the label.

Vial/Bottle	Label	Storage
1	LightCycler® Fluorescein CPG	Store the product at −15 to −25°C. ⚠ ***Store dry and protected from light.***

1.3. Additional Equipment and Reagents Required

For Oligonucleotide Labeling

- Standard reagents for oligonucleotide synthesis (tetrazol, etc.)
- 30 – 33% ammonia solution
- DNA synthesizer
- Vacuum centrifuge
- 0.45 µm membrane filter

For Oligonucleotide Purification by HPLC

- HPLC
- Mono Q separation medium (Amersham Pharmacia Biotech)
  This separation medium is recommended to obtain optimal purification results.
- 0.45 µm membrane filter
- Sodium hydroxide (10 mM; pH approx.12)
- Sodium chloride (1 M)
- Photometer

For Quality Control of HPLC-purified Oligonucleotides

- Photometer
- 1 M sodium borate buffer (pH 8.5)

1.4. Application

LightCycler® Fluorescein CPG labels the 3' end of an oligonucleotide that is combined with a 5' LightCycler® Red-labeled oligonucleotide when using HybProbe probes for sequence-specific detection in real-time PCR.
This support can be used in the same manner as standard nucleoside supports since it contains a dimethoxytrityl group.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Oligonucleotides, 20 – 30 bp in length.

General Considerations

Background Information

The LightCycler® Fluorescein is derived from a pure dye isomer and is attached via a linkage that is stable during oligonucleotide synthesis. Therefore, HPLC purification is simple and results in a well-defined product. HybProbe probes synthesized by using LightCycler® Fluorescein CPG are blocked at the 3'-terminus which prevents them from extension and exonuclease digestion by Taq DNA Polymerase. Thus, the resulting oligonucleotide is perfectly suited for single- and dual-color experiments using the LightCycler® Carousel-Based System.

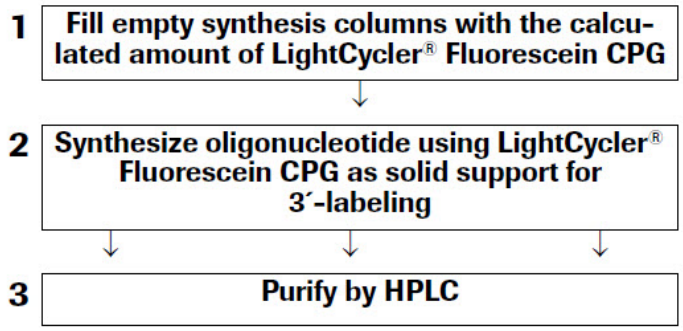
We recommend using the LightCycler® Probe Design Software 2.0 to design HybProbe probes and compatible PCR primers.

Labeling the Downstream HybProbe Probe

Label the 5' end of the downstream HybProbe probe with a LightCycler® Red fluorophore (*e.g.,* this can be done by post-labeling using LightCycler® Red 610 NHS ester* or LightCycler® Red 640 NHS ester*. Refer to the respective Instructions for Use for the detailed procedure).

2.2. Protocols

Flowchart



Filling the Synthesis Columns

The loading of the CPG material (µmol/g) is printed on the label. Depending on the desired synthesis scale, fill the respective amount of LightCycler® Fluorescein CPG into a commercially available empty synthesis column.

Filling Procedure

Fill the empty synthesis column as described below.

1. Calculate the amount of LightCycler® Fluorescein CPG needed by using the following formula (in mg):
   1/loading of CPG material (µmol/g) × synthesis scale (µmol) × 1,000 = amount needed (mg)

2. Fill the calculated amount into the empty synthesis column.

3. Tightly seal the filled column according to the manufacturer's recommendations.

Oligonucleotide Synthesis using LightCycler® Fluorescein CPG as Solid Support for 3'-Labeling

The oligonucleotide is modified at the 3' end with fluorescein during oligonucleotide synthesis. Introduce the 3'-fluorescein label by starting the oligonucleotide synthesis with LightCycler® Fluorescein CPG. This support contains a dimethoxytrityl-protected hydroxyl function that reacts with the first nucleoside phosphoramidite of the oligonucleotide synthesis cycle after deprotection.

Synthesis Procedure

Perform the oligonucleotide synthesis reaction as described below. Refer to the manufacturer's recommendations for the use of the respective reagents.

The following procedure describes a 0.2 µmol-scale oligonucleotide synthesis reaction and serves as an example for the application of LightCycler® Fluorescein CPG.

1. Connect the DNA synthesizer with a LightCycler® Fluorescein CPG column (0.2 µmol scale).

2. Program the synthesizer by entering an arbitrary base at the 3'-terminus of the oligonucleotide. The 3'-terminal base of the oligonucleotide sequence should be entered as the second base.
   – Set program to "Trityl off".
   – Start oligonucleotide synthesis.

3 Deprotect the oligonucleotide after cleavage with 30 – 33% ammonia solution from the CPG support, according to the protocol for standard phosphoramidite (50 – 55°C within 8 hours).

4 Filter the solution by using a 0.45 µm membrane filter.

5 Evaporate the solution under vacuum.

6 Store the remainder at −15 to −25°C.

HPLC Purification

Perform HPLC to separate the labeled oligonucleotide from unlabeled oligomers and impurities. Perform the purification of the labeled oligonucleotide as described below.

1 Dissolve the oligonucleotide (from section **Oligonucleotide Synthesis using LightCycler® Fluorescein CPG as Solid Support for 3'-Labeling**) in 1 ml double-distilled water.
– Filter the solution using a 0.45 µm membrane filter.
– Apply on a Mono Q column.

2 HPLC conditions are as follows:

Buffer	Description
Buffer A	Sodium hydroxide (10 mM, pH approx. 12)
Buffer B	1 M sodium chloride dissolved in sodium hydroxide (10 mM, pH approx. 12)
Gradient	In 30 minutes from 100% Buffer A to 100% Buffer B
Flow	1 ml/min
Detection	At 260 nm

A typical HPLC elution profile is shown in Figure 1.

3 Start the gradient after 2 minutes.

4 A small first peak appears at 50% of Buffer B which corresponds to a minor nonspecific impurity.

5 At 70 – 80% of Buffer B, further peaks appear which correspond to labeled n-x mers.

6 At about 85% of Buffer B, the main peak appears which corresponds to the desired product.

i According to the principle of IEX chromatography, the last peak always corresponds to the full-length product.

7 Collect the fraction from the main peak (Step 6).

8 Continue up to 100% Buffer B.

9 Purge column for 3 minutes with 100% of Buffer B for regeneration.

10 Desalt the solution from Step 7 by gelfiltration or dialysis.
– Lyophilize the desalted solution.

11 Store the pellet at −15 to −25°C.

HPLC Elution Profile

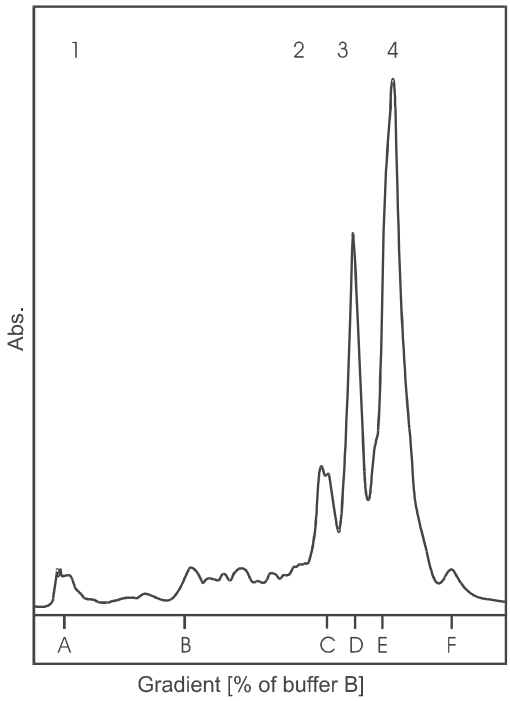


Fig. 1: A typical HPLC elution profile of a purification run of a 27-mer is shown. Peak 1 represents nonspecified impurities, peaks 2 and 3, labeled shortmeres, and peak 4, the 3'-labeled oligonucleotide (slightly yellow solution). The letters (A - F) indicate:
A: Start gradient
B: Gradient at 50% of Buffer B
C: Gradient at 75% of Buffer B
D: Gradient at 80% of Buffer B
E: Gradient at 85% of Buffer B and
F: 100% of Buffer B

Quality Control of HPLC-Purified Oligonucleotides

Characterize the 3'-labeled oligonucleotide by its UV/VIS absorption spectrum in the 200 – 600 nm range. Calculate the yield of labeled oligonucleotide by measuring the $A_{260\ nm}$ units.

Procedure to Determine the Yield of the Labeled Oligonucleotide

1. Dissolve the pellet (from section **HPLC Purification**) in 1 ml double-distilled water.
   – Add 40 µl of the solution to 760 µl 1 M sodium borate buffer, pH 8.5, in a cuvette.
   – Measure the extinction at 260 nm.

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2. Multiplying the extinction value by a factor of 20 gives the yield in $A_{260\ nm}$ units (one $A_{260\ nm}$ unit corresponds to approx. 5 nmol 20-mer oligonucleotide).

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3. Store the pellet at −15 to −25°C.

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4. Run a UV/VIS absorption spectrum in the 200 – 600 nm range. The resulting spectrum corresponds to Figure 2.

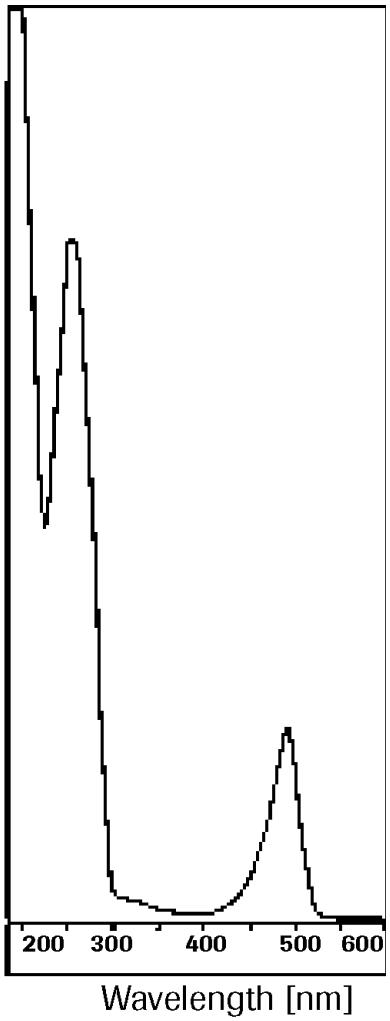

Fig. 2: UV/VIS absorption spectrum

5 Based upon the UV/VIS absorption spectrum, calculate the ratios of the extinction values at 495 nm and 260 nm. Depending on the length and sequence of the oligonucleotides, the approximate values are shown below:

Length of Oligonucleotide	Ratio ($A_{495\ nm}/A_{260\ nm}$)
20-mer	0.30 – 0.40
25-mer	0.25 – 0.35
30-mer	0.20 – 0.30

Labeling Efficiency

When performing the 0.2 µmol-scale oligonucleotide synthesis, the yield of the purified labeled oligonucleotide is approx. 10 – 25%, which corresponds to 20 – 50 nmols for a 20-mer.

3. Additional Information on this Product

3.1. Test Principle

Spectral Characteristics

An oligonucleotide labeled with LightCycler® Fluorescein shows an excitation maximum at 494 nm and an emission maximum at 519 nm (both measured in 2 mM Tris buffer, pH 8.3).

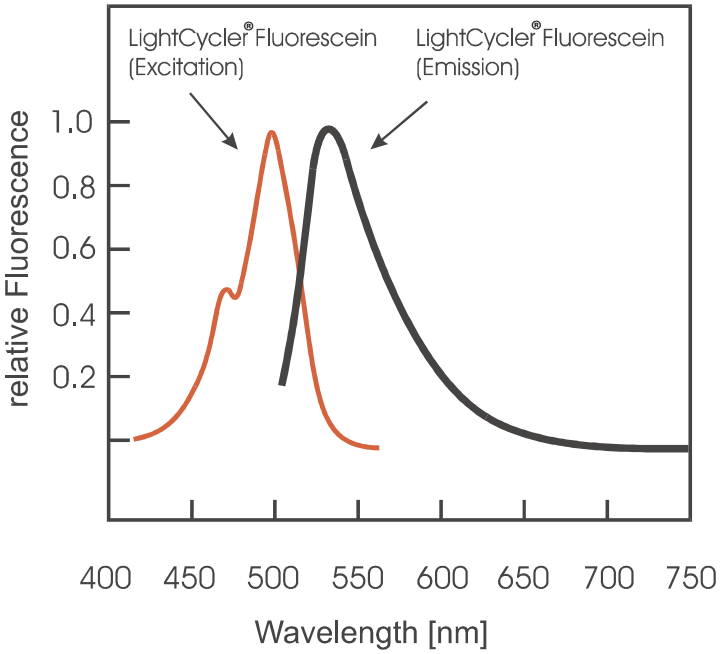

Fig. 3: Excitation and emission spectra of an oligonucleotide which is labeled with LightCycler® Fluorescein.

Labeling Principle

LightCycler® Fluorescein CPG contains a dimethoxytrityl-protected hydroxyl function which makes it suitable for elongation with standard phosphoramidites after conventional deprotection dring the first synthesis cycle.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® Software 4.1	1 software package	04 898 915 001
Accessories software		
LightCycler® Probe Design Software 2.0	1 software package	04 342 054 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
Reagents , kits		
LightCycler® Red 640-N-hydroxysuccinimide ester	1 vial, for 5 x 50 nmol oligonucleotides	12 015 161 001
LightCycler® Red 610-N-hydroxysuccinimide ester	1 vial, for 5 x 50 nmol oligonucleotides	03 561 488 001
LightCycler® Color Compensation Set	1 set, 4 vials, 5 calibration runs	12 158 850 001

4.4. Trademarks

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4.5. License Disclaimer

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4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

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