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Not for use in diagnostic procedures.

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SimpleProbe 519 Labeling Reagent

Version: 04
Content version: August 2017

For the synthesis of labeled oligonucleotides

Cat. No. 04 687 132 001	100 µmol For preparation of 10 internally labeled oligonucleotides (0.2 µmol scale)

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	SimpleProbe 519 Labeling Reagent	▪ Contains SimpleProbe 519 Labeling Reagent. ▪ The reagent is supplied as a yellow solid filled in glass bottles adaptable to oligonucleotide synthesizers.	1 bottle, 100 µmol

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	SimpleProbe 519 Labeling Reagent	Store at −15 to −25°C. ⚠ ***Store dry and protected from light.***

1.3. Additional Equipment and Reagents Required

For Preparation of the SimpleProbe 519 Labeling Reagent Solution

- Syringe/needle
- Anhydrous acetonitrile

For Oligonucleotide Labeling and Phosphorylation

- DNA synthesizer
- Vacuum centrifuge
- Standard reagents for oligonucleotide synthesis (tetrazole, etc.)
- 30 – 33% ammonia solution
- 3'-Phosphate-CPG-support
- N6-Bz-dA-Phosphoramidite
- N2-iBu-dG-Phosphoramidite
- N4-Bz-dC-Phosphoramidite
- T-Phosphoramidite

For Oligonucleotide Purification by HPLC

- HPLC
- Strong anion exchange material (for example, Mono Q HR, or Source 15 Q). This separation medium is recommended to obtain optimal purification results.
- Photometer
- Sodium hydroxide (10 mM; pH ~12)
- Sodium chloride (1 M)

For Quality Control of HPLC-Purified Oligonucleotide

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

1.4. Application

SimpleProbe 519 Labeling Reagent is used for internal labeling of a mutation detection probe, which could be used in the SimpleProbe detection format with the respective LightCycler® System.

2. How to Use this Product

2.1. Protocols

Oligonucleotides react with the SimpleProbe 519 Labeling Reagent during the solid phase phosphoramidite synthesis method. This reagent can be used in the same manner as standard phosphoramidite (with 2 minutes coupling time) since it contains a dimethoxytrityl group. The reagent is stable during oligonucleotide synthesis. Therefore, HPLC purification is simple and results in a well-defined product.

Workflow Overview

The entire procedure for generating a labeled SimpleProbe probe using the SimpleProbe 519 Labeling Reagent includes the following steps:

① Preparation of SimpleProbe 519 Labeling Reagent Solution.

② Synthesis of the oligonucleotide including internal labeling and 3'-end phosphorylation:

Labeling	Protocol
3' end	Introduction of 3'-phosphate group by starting the oligonucleotide synthesis with a corresponding modified CPG support.
Internally	Labeling reaction with SimpleProbe 519 Labeling Reagent in a defined synthesis cycle in order to obtain a mutation detection probe labeled at a position according to the design rules.

③ IEX-HPLC to separate the labeled oligonucleotide from unlabeled oligomeres and impurities.

④ Quality control of the purified oligonucleotide by measuring the UV/VIS absorption spectrum in the 200 – 600 nm range.
– Calculation of the yield of labeled oligonucleotide by measuring the A_{260} units.

Preparation of SimpleProbe 519 Labeling Reagent Solution

❶ Inject 1.0 ml anhydrous acetonitrile into the sealed SimpleProbe 519 Labeling Reagent bottle using a syringe with a needle.

❷ Dissolve the SimpleProbe 519 Labeling Reagent solution by swirling until all reagents have dissolved.
– If necessary, filter the obtained solution with 0.45 µm membrane syringe filter (the membrane must be stable to organic solutions).

❸ Remove the needle and apply the bottle to the DNA synthesizer.

If the bottle is not directly attachable, withdraw the solution into the syringe and transfer the solution to the appropriate reservoir on the synthesizer.

Oligonucleotide Synthesis, Phosphorylation, and Labeling

1. Connect the DNA synthesizer with a 3'-phosphate CPG column.
   – Use a 0.2 µmol phosphate CPG support.

2. Depending on the synthesizer used, attach the bottle with the SimpleProbe 519 Labeling Reagent solution (see section **Preparation of SimpleProbe 519 Labeling Reagent Solution**) to the respective position, or transfer the solution to the appropriate reservoir.

3. Program the synthesizer by entering an arbitrary base at the 3'-terminal base of the oligonucleotide. The 3'-terminal base of the oligonucleotide sequence should be entered as the second base.
   – Enter the desired sequence and add as the labeling cycle, the position where the bottle with SimpleProbe 519 Labeling Reagent solution is attached. Extend the coupling time to 2 minutes for the SimpleProbe 519 Labeling Reagent.
   – Set program to "Trityl off".
   – Start oligonucleotide synthesis.

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

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

6. Evaporate the solution under vacuum.

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

HPLC Purification

1. Dissolve the oligonucleotide (from section **Oligonucleotide Synthesis, Phosphorylation, and 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:

Parameter Condition	
Buffer A	Sodium hydroxide (10 mM; pH ~12)
Buffer B	1 M sodium chloride dissolved in sodium hydroxide (10 mM; pH ~12)
Gradient	In 50 minutes from 30% Buffer B to 100% Buffer B
FLOW	1 ml/min
Detection	At 260 nm

i A typical HPLC elution profile is shown in Figure. 1.

3. Start the gradient after 2 minutes.

4. A small first peak appears at 34% of Buffer B which refers to a minor nonspecific impurity.

5. At 50 – 58% of Buffer B, further peaks appear which refer to labeled n-x meres.

6 At about 62% of Buffer B, the main peak appears which refers to the desired product.

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

7 Collect 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

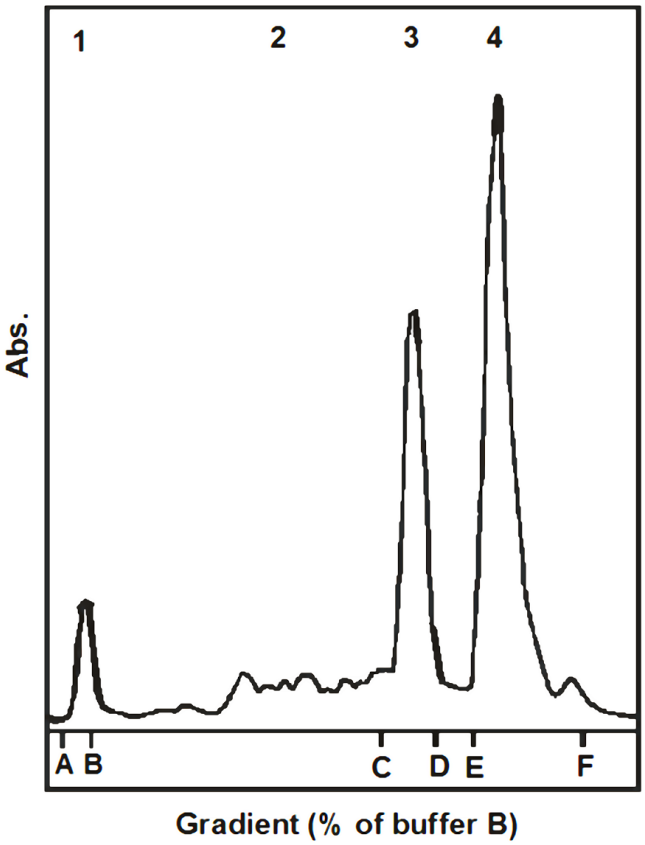

Fig. 1: Typical HPLC elution profile of a 22-mer oligonucleotide. Peak 1 represents non-specified impurities, peaks 2 and 3 represent shortmeres, and peak 4, the internally labeled oligonucleotide (slightly yellow solution). The letters (A-F) indicate:

A: Start gradient at 30% of Buffer B
B: Gradient at 35% of Buffer B
C: Gradient at 50% of Buffer B
D: Gradient at 58% of Buffer B
E: Gradient at 62% of Buffer B
F: 100% of Buffer B

Quality Control of HPLC-Purified Oligonucleotides

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

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

   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. Store the pellet at −15 to −25°C.

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

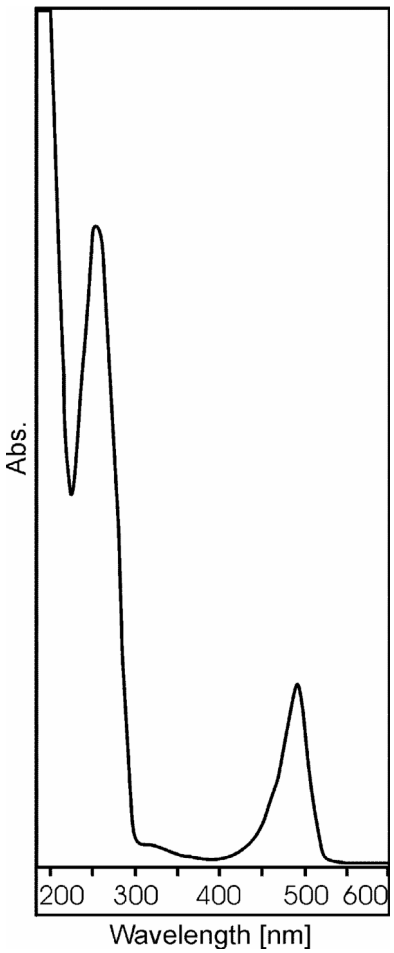

Fig. 2: UV/VIS absorption spectrum.

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

Oligonucleotide Length	Ratio (A_{495}/A_{260})
20-mer	0.20 – 0.30
25-mer	0.17 – 0.20
30-mer	0.15 – 0.17

3. Additional Information on this Product

3.1. Test Principle

SimpleProbe chemistry uses a single, fluorescently labeled probe that is designed to hybridize to a target sequence containing the SNP of interest. Immediately following PCR, amplified product is denatured and rapidly cooled, allowing the SimpleProbe probe to hybridize to its target-specific sequence. Once hybridized, the SimpleProbe probe emits a greater fluorescent signal than it does when it is not hybridized to its target. At low temperatures, the fluorescent signal from the hybridized probes is high due to the large number of probes hybridized to their target sequence. As the temperature of the reaction is slowly increased, the SimpleProbe melts at the Tm of the probe, causing the fluorescent signal to decrease. Thus, changes in fluorescent signal depend solely on the hybridization status of the probe. Mutations, such as SNP, weaken the binding of the SimpleProbe probe, causing the probe to melt off at a lower Tm compared to the wild type sequence. Therefore, SimpleProbe probes are an excellent tool for SNP genotyping and mutation detection because they readily identify wild type, mutant, and heterozygous samples with only a single short probe.
Please find the LightCycler® Probe Design Software 2.0 on our website for designing optimized PCR primers and probes for a given DNA sequence.

Spectral Characteristics

An oligonucleotide internally labeled with SimpleProbe 519 Labeling Reagent shows an excitation maximum at 494 nm and an emission maximum at 519 nm (both measured in 50 mM Tris, pH 8.3).

⚠ ***The 3' end of the probe has to be phosphorylated to avoid elongation during PCR.***
⚠ ***Recommended length of oligonucleotides is 20 – 30 nt.***

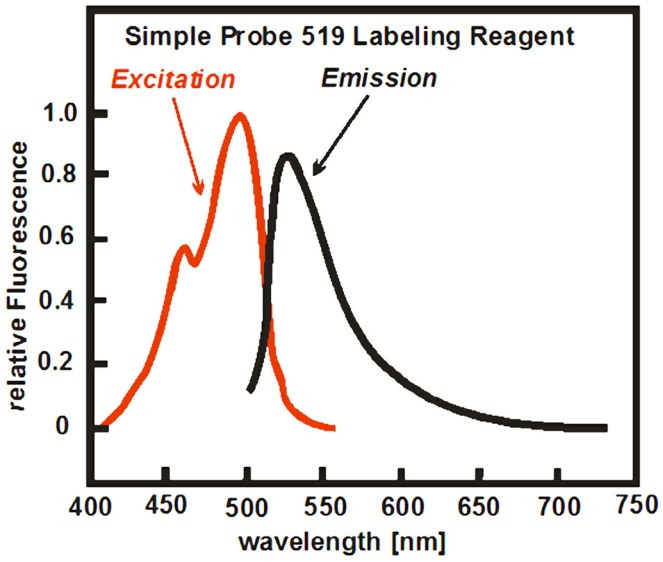

Fig. 3: Excitation and emission spectra of an oligonucleotide, labeled internally with SimpleProbe 519 Labeling Reagent.

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.
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001

4.4. Trademarks

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4.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

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

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
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