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

Fluorescein-High Prime

For the nonradioactive labeling of DNA with fluorescein-12-dUTP using random oligonucleotides as primers
Premixed solution for 25 labeling assays

Cat. No. 11 585 622 910

100 µl

Version 07
Content version: July 2019

Store at −15 to −25°C

1. Product overview

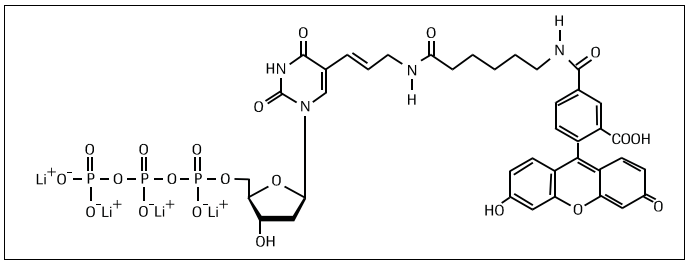
Contents

Label	Content
Fluorescein-High Prime labeling mixture, 5 × conc.	• 100 µl • 5 × conc.: random primer mix, 1U/µl Klenow polymerase, labeling grade, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM fluorescein-12-dUTP, and 5 × stabilized reaction buffer in 50% (v/v) glycerol

Labeling principle

Fluorescein-labeled DNA probes are generated with Fluorescein-High Prime according to the "random primed" labeling technique (1,2). The complementary DNA strand is synthesized by Klenow polymerase using the 3'OH termini of the random oligonucleotides as primers. Fluorescein-12-dUTP (Fig. 1) is incorporated into the newly synthesized complementary DNA strand.
Fluorescein-High Prime is a specially developed reaction mixture containing random oligonucleotides, Klenow polymerase, fluorescein-12-dUTP, dATP, dCTP, dGTP, dTTP, and an optimized reaction buffer concentrate in 50% glycerol for convenient and efficient nonradioactive labeling of DNA with fluorescein.

Figure 1: Structure of fluorescein-12-dUTP



Application

Fluorescein-High Prime labeled probes are used in a variety of hybridization techniques:

- Southern blots (3),
- Dot/slot blots
- screening of gene libraries (4),
- *in situ* hybridizations.

Sample material

- DNA fragments of at least 100 bp,
- linearized plasmids, cosmid or λDNA,
- supercoiled DNA,
- or minimal amounts of DNA (10 ng), e.g. DNA restriction fragments isolated from gels or in molten agarose can be used.

Number of labeling reactions

25 labeling reactions containing 0.01-3 µg DNA can be performed with Fluorescein-High Prime.

Storage/Stability

The unopened vial is stable at -15 to -25° C until the expiration date printed on the label.
Note: Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot the Fluorescein-High Prime solution and to store in 2-3 portions.

Detection of fluorescein-labeled DNA

Fluorescein-labeled DNA is detected by:

- a polyclonal antibody alkaline phosphatase conjugate from sheep (anti-fluorescein AP, Fab fragments*) which catalyzes a color reaction with 5-bromo-4-chloro-3-indolylphosphate and 4-nitro blue tetrazoliumchloride* (NBT/BCIP)
- or a chemiluminescent reaction with CSPD* or CDP-Star*.

For *in situ* applications, fluorescein-labeled hybrids can also be detected directly by fluorescence microscopy or

- the fluorescent signal can be enhanced by using different combinations of primary and secondary fluorescein-labeled antibodies, e.g., a monoclonal anti-fluorescein antibody*.

2. Procedures and required material

2.1 Standard labeling assay

Additional equipment and reagents required

- water bath
- ice/water
- 0.2 M EDTA (pH 8.0)

Procedure

In the following table please find a protocol for the standard labeling assay.

Step	Action
1	Add 1 µg **template DNA** (linear or supercoiled) and sterile, double dist. water to a final volume of 16 µl to a reaction vial.
2	Denature the DNA by heating in a boiling water bath for 10 min and chilling quickly in an ice/water bath. ***Note:*** Complete denaturation is essential for efficient labeling.
3	• Mix **Fluorescein-High Prime** thoroughly and add 4 µl to the denatured DNA, mix, and centrifuge briefly. • Incubate for 1 h or O/N at +37° C. ***Note:*** Longer incubations (up to 20 h) will increase the yield of Fluorescein-labeled DNA (see table below).
4	Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to +65° C for 10 min. ***Note:*** The length of the Fluorescein labeled fragments obtained with Fluorescein-High Prime range from 200 bp to 1000 bp or larger, depending on the lengths of the original template.

2.2 Labeling assay using low-melting point agarose

Procedure

In the following table the procedure for the labeling of DNA isolated from low-melting point agarose is described.

Step	Action
1	Excise the **DNA fragment** to be labeled cleanly from a low-melting point agarose gel and transfer it to a 1.5 ml microfuge tube.
2	• Add **sterile, double dist. water** to a ratio of 3 ml/g gel and heat the tube for 7 min at +100°C to melt the gel and denature the DNA. • After cooling to +37° C the DNA/agarose mixture can be used directly for labeling
3	Mix **Fluorescein-High Prime** thoroughly and add 4 µl, mix, and centrifuge briefly.
4	Incubate overnight at +37° C.
5	Stop the reaction by adding 2 µl 0.2 M **EDTA** (pH 8.0) and/or by heating to +65° C for 10 min.

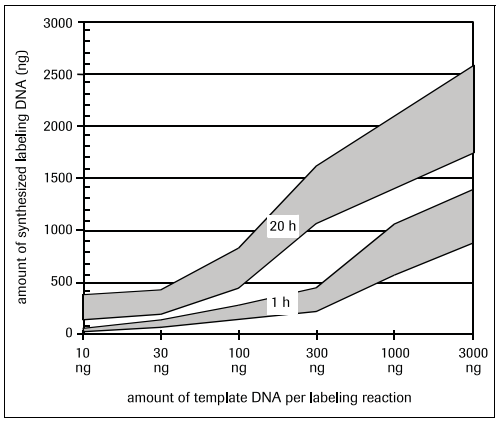
3. Efficiency of Fluorescein-High Prime Labeling

Table 1 **Yield of Fluorescein-High Prime labeling reaction**

Using the Fluorescein-High Prime solution labeling reactions were performed with increasing amounts of different template DNAs for 1 h and 20 h. The yield of fluorescein-labeling DNA was determined by the incorporation of a radioactive tracer and confirmed by a dot blot. (Average of independent labeling assays).

Template DNA	1 h	20 h
10 ng	30 ng	250 ng
30 ng	70 ng	320 ng
100 ng	200 ng	650 ng
300 ng	320 ng	1350 ng
1000 ng	850 ng	1700 ng
3000 ng	1200 ng	2100 ng

Figure 2: Yield of fluorescein-labeled DNA from different amounts of template DNAs for 1 h and 20 h incubation of the Fluorescein-High Prime reaction at +37°C.



4. Appendix

4.1 References

1 Feinberg, A.P. & Vogelstein, B.(1983) *Anal. Biochem.* **132**, 6.
2 Feinberg, A.P. & Vogelstein, B.(1984) *Anal. Biochem.* **137,** 266.
3 Southern, E.M. (1975) *J. Mol. Biol.* **98,** 503.
4 Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961.

Changes to previous version

- Editorial changes.

4.2 Ordering Information

Kits/Sets

Product	Pack Size	Cat. No
DIG-High Prime DNA Labeling and Detection Starter Kit II	1 kit (12 labeling reactions and 24 detection reactions)	11 585 614 910
DIG Wash and Block Buffer Set	30 blots (10×10 cm^2)	11 585 762 001

Single reagents

Product	Pack Size	Cat. No.
Anti-fluorescein AP, Fab fragments	150 U	11 426 338 001
Anti-fluorescein antibody	100 µg	11 426 320 001
Biotin-High Prime	100 µl	11 585 649 910
CSPD, ready-to-use	2×50 ml	11 755 633 001
CDP-*Star*, ready-to-use	2×50 m	12 041 677 001
DIG-High Prime	160 µl	11 585 606 910
High Prime	200 µl	11 585 592 910
Hybridization bags	50 bags	11 666 649 001
Klenow Enzyme	100 units 500 units	11 008 404 001 11 008 412 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
Nylon Membrane, positively charged (20×30 cm) (10×15 cm) (0.3×3 m roll)	 10 sheets 20 sheets 1 roll	 11 209 272 001 11 209 299 001 11 417 240 001

Trademarks All third party product names and trademarks are the property of their respective owners.

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

Disclaimer of License For patent license limitations for individual products please refer to: List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany