Print

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

PCR Fluorescein Labeling Mix

For direct labeling of amplification products with fluorescein-dUTP in the polymerase chain reaction (PCR)

Cat. No. 11 636 154 910

Version 06
Content version: August 2006

Store at −15 to −25° C

Product description

The mix contains

Vial 1 PCR Fluorescein Labeling Mix (10 × conc.) for 10 PCR assays a 100 µl. The solution contains 2 mM dATP, dCTP, dGTP each; 1.5 mM dTTP; 0.5 mM fluorescein-12-dUTP in 100 µl water, pH 7.0.

Vial 2 PCR reaction buffer (10 × conc) without $MgCl_2$; 1 ml 100 mM Tris-HCl, 500 mM KCl, pH 8.3 (20° C).

Vial 3 25 mM $MgCl_2$ stock solution, 1 ml

PCR Fluorescein Labeling Mix is a mixture of the lithium salts of dATP, dCTP, dGTP, dTTP and fluorescein-12-dUTP. This nucleotide mix can be added directly to polymerase chain reactions (1), in which the fluorescein-labeled nucleotide is incorporated into the PCR product. Taq DNA polymerase* as well as Tth DNA polymerase* can be used for the synthesis of fluorescein-labeled PCR products.

PCR Fluorescein Labeling Mix is especially useful for the synthesis of labeled probes, when only limited amounts of DNA are available. Fluorescein-labeled PCR products are mainly used as probes for *in situ* hybridization. Detection of fluorescein-labeled DNA on membranes is possible with anti-fluorescein-AP conjugate*.

The nucleotide concentration in PCR Fluorescein Labeling Mix is optimized for the synthesis of sensitive fluorescein-labeled probes.

Stability

Stable at −15 to −25° C. The mix should be stored protected from light.

Application

PCR Fluorescein Labeling Mix can be used in the place of the unlabeled PCR Nucleotide Mix*. 10 µl PCR Fluorescein Labeling Mix is used in a 100 µl PCR standard assay. All kinds of template DNA can be used for the synthesis of fluorescein-labeled probes.
The amount of synthesized PCR product may be lower than in a PCR with an unlabeled nucleotide mix due to the high concentration of labeled nucleotide.

A higher labeling efficiency with the PCR Fluorescein Labeling Mix is observed when using a higher Mg^{2+} concentration compared to the unlabeled nucleotide mix. We recommend a standard concentration of 4 mM Mg^{2+} in the reaction mixture.

Composition of a PCR standard assay for the synthesis of fluorescein labeled PCR products is described below.

Optimal reaction conditions are dependent on template-DNA and primer. In particular, incubation times and temperatures, concentrations of Mg^{2+} and enzyme, as well as concentrations of template-DNA and primers should be optimized for optimal results for each new primers/template pair (2).

* available from Roche Applied Science

PCR standard assay for fluorescein labeling:

Add the following components to a sterile microcentrifuge tube. Place the tube on ice during pipetting.

Reagent	Volume	Concentration
Sterile redist. H_2O	variable	–
PCR buffer, 10 × without $MgCl_2$	10 µl	1 ×
$MgCl_2$ stock solution (25 mM)	16 µl	4 mM
PCR Fluorescein Labeling Mix	10 µl	200 µM dNTP
Primer 1	variable	0.1–1 µM
Primer 2	variable	0.1–1 µM
Taq DNA polymerase	0.2–1 µl	1–5 U
Template DNA	variable	variable
Total	**100 µl**	

Application example

In general any type of template DNA may be used for synthesis of fluorescein-labeled probes. As an example the synthesis of a probe specific for human alphoid sequences is described. Alphoid sequences (alpha satellites) are repetitive sequences of a 171 bp consensus monomer located at the centromere of all human chromosomes.

1. Probe synthesis with fluorescein PCR

A plasmid containing repetitive alphoid sequences from a human chromosome is used as template DNA. The primers are specific for alphoid consensus sequences.

Procedure

PCR-assay

10 pg plasmid DNA
200 ng of each PCR primer
10 µl PCR Fluorescein Labeling Mix
10 µl 10 × PCR reaction buffer (without $MgCl_2$)
16 µl 25 mM $MgCl_2$ stock solution
2.5 U Taq DNA polymerase
reaction volume 100 µl

Cycling conditions

4 min at +95° C before the first cycle
30 cycles: 45 s at +95° C (denaturation)
1 min at +60° C (annealing)
2 min at +72° C (elongation)
5 min at +72° C after the last cycle

Amplification product

PCR fragments consisting of multimeres of the repetitive sequence.
The labeled probe should be stored at −15 to −25° C protected from light. It is stable for up to 6 months.

2. Detection of fluorescein-labeled PCR products on membranes

Detection is performed using anti-fluorescein-AP conjugate and colorimetric detection.

Alternatively, the chemilumescent substrate CSPD may be used with subsequent exposure of the blot to X-ray film or imaging instrument.

- Spot an aliquot of the PCR assay in a dilution series on a nylon membrane, positively charged*.
- Incubate the membrane with anti-fluorescein-AP conjugate (150 mU/ml diluted in blocking reagent*, 1% (w/v), 100 mM maleic acid, 150 mM NaCl, pH 7.5 (20°C) [buffer 2]) for 30 min at +15- to +25° C.
- Wash two times for 15 min with 100 mM maleic acid, 150 mM NaCl, pH 7.5 (+20° C) [buffer 1].
- Equilibrate for 2 min in 100 mM Tris-HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5 (+20° C) [buffer 3].
- Add NBT/BCIP* or chemiluminescent AP-substrates* to visualize the signal.

3. *in situ* hybridization

The fluorescein-labeled probe against alphoid sequences is hybridized against human metaphase spreads:

Additional required reagents and solutions for *in situ* hybridization

- Human metaphase chromosomes
- 20 × SSC
- Ethanol, 100%
- PBS*
- Tween 20*
- Formamide, deionized
- 4 × hybridization mix: 8 × SSC; 40% dextrane sulfat, 4 mg/ml DNA, MB grade,
- Fixogum
- Propidium iodide, 1 mg/ml in H_2O
- Antifading reagent

Hybridization protocol

- Lyophilize 2–5 µl of the PCR reaction volume in a speed vac.
- Dissolve the labeled DNA in 20 µl hybridization solution composed of 5 µl hybridization buffer (8 × SSC, 40% (w/v) dextrane sulphate, 4 mg/ml DNA MB grade*), 50%–70% deionised formamid and bidest, H_2O.
- Spot the hybridization solution on slides with human metaphase chromosomes.
- Cover by a cover slip (22 × 22 mm) and seal with Fixogum.
- Denature for 2.5 min at +72° C (*e. g.* on a pre-warmed glass plate in the oven).
- Hybridize the slide overnight at +37° C in a moist chamber.
- Wash the slides once for 15 min in 50% formamide / 1 × SSC at +42 to +48° C.
- Wash three times for 5 min in 0.1 × SSC at +60°C and three times for 5 min in PBS/0.2% Tween 20 at +37° C.
- Incubate the slides for counter staining in 60 ml PBS + 1 µl propidium iodide (1 mg/ml) for 5 min at +15 to +25° C in the dark and wash briefly in H_2O.
- Dehydrate the slides by washing in a series of increasing ethanol concentration (80%, 90% and 100%) for 3 min each.
- Air dry in the dark.
- Spot 20 µl antifading solution on the slide (22 × 22 mm) and cover by a cover slip.
- Detect the signals by fluorescence microscopy. Use an appropriate filter for detection: emission wavelength for fluorescein is 523 nm.

Quality control

PCR Fluorescein Labeling Mix is function tested in PCR. Amplification products are assayed by dot blot and in *in situ* hybridization experiments. For the dot blot analysis the fluorescein PCR assay is spotted in a dilution series on a nylon membrane and detected with anti-fluorescein-AP as described above. Detection limit is at least 10^{-3} µl PCR reaction volume. For *in situ* hybridization alphoid sequences in human chromosomes are detected as described above.

PCR Fluorescein Labeling Mix is free of DNases and RNases.

References

1 Saiki, R. et al. (1985) Science 230, 1350–1354.
2 Rolfs, A. et al. (1992) PCR: Clinical Diagnostics and Research, Springer Verlag, Berlin.

Please refer to our website for the following informations:
3 Non-radioactive In situ Hybridization Manual

Changes to previous version Regulatory disclaimer updated

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

Ordering Information Roche Applied Science offers a large selection of enzymes, reagents, and systems for PCR and RT-PCR assays. For a complete overview of our products and for more detailed information on PCR and RT-PCR please visit and bookmark our Amplification Special Interest Site at http://www.roche-applied-science.com/PCR.

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Instructions for Use and Material Safety Data Sheets.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany