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PCR DIG Labeling Mix

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

Cat. No. 11 585 550 910

2× 250 µl

Version 09

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Store at −15 to −25° C

Product overview

Contents

Solution, 10× conc.

PCR DIG labeling mix is a mixture of the sodium salts of dATP, dCTP, dGTP, dTTP and digoxigenin-11-dUTP, lithium salt.

The solution contains 2 mM dATP, dCTP, dGTP each, 1.9 mM dTTP, 0.1 mM digoxigenin-11-dUTP (DIG-11-dUTP) in 2 × 250 µl water; pH 7.0.

Description

This nucleotide mixture can be added directly to polymerase chain reactions (1) and the DIG-(digoxigenin-) labeled nucleotide will be incorporated into the PCR product (2). Taq DNA polymerase, as well as Tth DNA polymerase, can be used for the synthesis of DIG-labeled PCR products.

The PCR DIG labeling mix can replace the unlabeled nucleotide mix in PCR. 10 µl of the PCR DIG labeling mix is used in a standard 100 µl PCR assay. Composition and cycling conditions of a standard PCR assay for the synthesis of DIG-labeled PCR products are described in the table below (see also documentation of Taq DNA polymerase*). A detailed example is given under quality control.

Application

The PCR DIG labeling mix is especially designed for the sensitive detection of PCR products and for the sensitive analysis of PCR reactions.

At higher concentrations of DIG-dUTP than supplied with the PCR DIG labeling mix the yield of the PCR product may be reduced, but the label intensity and therefore also the molecular weight of the PCR product is increased.

PCR DIG labeling mix can also be used for the synthesis of hybridization probes. However for the production of highly sensitive probes, for example necessary to detect single copy genes on genomic blots, we recommend a PCR nucleotid mix with an increased concentration of DIG-dUTP (*e.g.,* PCR DIG Probe Synthesis Kit*).

Number of reactions

For 2 × 25 PCR assays per 100 µl (final concentration 200 µM).

Quality control

The PCR DIG labeling mix is function-tested in PCR.

Amplification products are assayed by dot blot and in hybridization experiments (see below). The PCR DIG labeling mix is free of DNases and RNases according to current quality control procedures.

Storage/Stability

Stable at -15 to -25°C until the control date printed on the label.

Advantages

The nucleotide concentration in the PCR DIG labeling mix ensures:

1. no limitations of the PCR reaction resulting from shortage of nucleotides,

2. maximum yield of PCR product combined with sensitive DIG-detection,

3. only minor influence on the molecular weight of the product,

4. DIG labeling in PCR with an economical concentration of DIG-dUTP.

** available from Roche Applied Science*

Procedures and required material

Before you begin

Optimal reaction conditions are dependent on template DNA and primer. In particular incubation times and temperatures, concentration of Mg^{2+} and enzyme but also concentration of template DNA and primer should be optimized for optimal results for each new primer/template pair (3).

Additional reagents required

- Sterile double dist. water*
- PCR buffer, 10× conc.,15 mM $MgCl_2$*
- Taq DNA Polymerase, 5 U/µl*
- Primer
- Template DNA

Procedure

Please refer to the following table.

Step	Action
1	Add the following components to a sterile microcentrifuge tube on ice:
2	Mix the reagents and centrifuge briefly to collect the sample at the bottom of the tube.
3	Overlay with 100 µl mineral oil to reduce evaporation of the mix during amplification. ***Note:*** If your thermal cycler has a top heater, the oil overlay is not necessary.
4	Place samples in the thermal cycler and start PCR. ***Note:*** When using the PCR DIG labeling mix PCR conditions are the same compared to conditons established for a defined primer/template pair.
5	The DIG-labeled PCR probe should be stored:

Reagent	Volume	Final conc.
Sterile double dist. water	variable	
PCR buffer, 10× conc., 15 mM $MgCl_2$	10 µl	1,5 mM $MgCl_2$
PCR DIG labeling mix	10 µl	200 µM dNTPs
Primer 1	1 µl	0.1-1 µM
Primer 2	1 µl	0.1-1 µM
Taq DNA Polymerase	0.2-1 µl	1-5 U/100 µl
Template DNA	variable	variable
Total volume	**100 µl**	

Short term	At +2 to +8°C until the PCR product is used for hybridization.
Long term	At -15 to -25°C, stable for at least one year.

General remarks on usage and application

Detection of PCR products with increased sensitivity

Due to the high sensitivity of the DIG system PCR products can be visualized which are not detectable with conventional ethidium bromide staining.

In particular this is advantageous, if only small amounts of template DNA are available for amplification. Furthermore side products of any PCR reaction can be detected with high sensitivity due to the incorporated DIG label. The PCR product is separated by agarose gel electrophoresis and blotted to a nylon* or nitrocellulose membrane. The incorporated DIG label is detected by using anti-digoxigenin-AP conjugate* and colorimetric detection with the substrate NBT/BCIP. Alternatively the chemiluminescent substrates CSPD/ CDP-Star can be used with subsequent exposure to an X-ray film or imaging instrument.

These methods are described in detail in the documentation for the

- DIG Nucleic Acid Detection Kit and the
- DIG Luminescent Detection Kit for Nucleic Acids.

Probe synthesis

The PCR DIG labeling mix is designed for the synthesis of DIG-labeled hybridization probes with PCR. All kinds of template DNA can be used for probe synthesis. The amount of labeled PCR product that has to be used for hybridization depends on the following parameters:

- format of hybridization
- targeted sensitivity
- efficiency of the PCR reaction
- primer/template pair

Usually about 10 µl of the PCR reaction per 1 ml of hybridization solution will yield good results.

Note: Especially when using genomic DNA as template, side products may be observed. These products will also become DIG-labeled during PCR. In hybridizations those side products may cause a higher background or even false positive signals.

Quality Control

Polymerase chain reaction

Template DNA	5 kb plasmid containing the cDNA for human tissue type plasminogen activator (tPA)
tPA primer	primer 1: AGA CAG TAC AGC CAG CCT CA primer 2: GAC TTC AAA TTT CTG CTC CTC
PCR-assay	1 pg plasmid DNA; 165 ng PCR primer 1; 165 ng PCR primer 2; 10 µl PCR DIG labeling mix; 10 µl 10x PCR reaction buffer (15 mM $MgCl_2$); 2.5 U Taq DNA polymerase; 100 µl reaction volume
Cycling conditions	1 cycle: Denaturation, 7 min, +95°C 30 cycles: Denaturation, 45 s, +95°C Annealing, 1 min, +60°C Elongation, 2 min, +72°C
Amplification product	PCR fragment of 264 bp

Dot blot

After spotting the PCR assay in a dilution series on a nylon membrane and detection with a DIG Nucleic Acid Detection Kit as little as 10 -5 µl of the PCR reaction can be detected.

Hybridization

The template-plasmid is spotted in a dilution series on a nylon membrane and hybridized with the DIG-labeled PCR fragment using 10 µl of the PCR reaction in 1 ml hybridization solution. The DIG label is detected with the DIG Nucleic Acid Detection Kit. At least 5 pg of the plasmid can be detected, which corresponds to 0.25 pg homologous DNA.

References

1 Saiki, R et al. (1985) *Science* **230**, 1350-1354.
2 Lion, T. & Haas, O.A. (1990) *Anal. Biochem.* **188**, 335-337.
3 Rolfs, A. et al. (1992) PCR: Clinical Diagnostic and Research, Springer Verlag, Berlin

Ordering Information

For further information:

Roche Applied Science offers a large selection of products for the non-radioactive labeling and detection of nucleic acids.For a complete overview, please visit and bookmark our "DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection" Special Interest Site at http://www.roche-applied-science.com/DIG and for PCR and RT-PCR related products please visit and bookmark our Amplification Special Interest site: http://www.roche-applied-science.com/PCR

Kits

Product	Pack size	Cat. No.
DIG Nucleic Acid Detection Kit	40 blots (10× 10 cm)	11 175 041 910
DIG Luminescent Detection Kit for Nucleic acids	1 kit (50 blots)	11 363 514 910
PCR DIG Probe Synthesis Kit	25 reactions	11 636 090 910

Single reagents

Product	Pack size	Cat. No.
Taq DNA Polymerase, 5 U/µl	10× 250 U 20× 250 U 100 U 500 U 4× 250 U	11 596 594 001 11 435 094 001 11 146 165 001 11 146 173 001 11 418 432 001
Water, PCR Grade	100 ml 25 ml 25 ml	03 315 843 001 03 315 932 001 03 315 959 001
Digoxigenin-11-dUTP, alkali-labile	25 nmol (25 µl) 125 nmol	11 573 152 910 11 573 179 910
Digoxigenin-11-dUTP, alkali-stable	25 nmol (25 µl) 125 nmol 5× 125 nmol	11 093 088 910 11 558 706 910 11 570 013 910
Anti-DIG-AP, Fab frag.	150 U (200 µl)	11 093 274 910
NBT/BCIP stock solution	8 ml	11 681 451 001
CDP-*Star*, ready -to-use	2× 50 ml	12 041 677 001
CSPD , ready-to-use	2× 50 ml	11 755 633 001
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

Changes to previous version

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Regulatory disclaimer updated

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