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

PCR DIG Labeling Mix

Version: 11
Content Version: June 2021

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

Cat. No. 11 585 550 910 2 x 250 µl
2 x 25 PCR assays of 100 µl final volume each

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	PCR DIG Labeling Mix, 10x conc.	dNTP labeling mixture: Sodium salts of 2 mM dATP, dCTP, dGTP each, 1.9 mM dTTP, 0.1 mM digoxigenin-11-dUTP (DIG-11-dUTP), lithium salt in water, pH 7.0.	2 vials, 250 µl each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	PCR DIG Labeling Mix, 10x conc.	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Autoclaved microcentrifuge tubes
- Standard benchtop microcentrifuge
- Thermal cycler

For standard labeling assay

- Water, PCR Grade*
- PCR Buffer Set*
- Taq DNA Polymerase, 5 U/µl*
- Primer
- Template DNA
- Mineral oil

1.4. Application

The PCR DIG Labeling Mix is especially designed for the:

- Sensitive detection of PCR products and 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, however the label intensity and therefore also the molecular weight of the PCR product is increased.
- Synthesis of hybridization probes.
  For the production of highly sensitive probes, for example, necessary to detect single-copy genes on genomic blots, use a PCR nucleotide mix with an increased concentration of DIG-dUTP, such as in the PCR DIG Probe Synthesis Kit.*

Product Description

This nucleotide mixture can be added directly to polymerase chain reactions and the DIG-labeled nucleotide will be incorporated into the PCR product. 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.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Optimization

Optimal reaction conditions depend on:
- Template DNA and primer.
  Optimize the concentration of the template DNA and primer for each new primer/template pair.
- Incubation times and temperatures.
- Concentration of Mg^{2}+ and enzyme.

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. This is especially advantageous if only small amounts of template DNA are available for amplification. Due to the incorporated DIG label, side products of any PCR can be detected with high sensitivity.

① PCR product is separated by agarose gel electrophoresis and blotted onto a Nylon* or nitrocellulose membrane.

② Incorporated DIG label is detected using Anti-Digoxigenin-AP conjugate* and colorimetric detection with the substrate NBT/BCIP*.

③ Alternatively, the chemiluminescent substrates CSPD* or CDP-*Star*® can be used with subsequent exposure to an imaging instrument, or X-ray film or Lumi-Film*.
These methods are described in detail in the Instructions for Use of the DIG Nucleic Acid Detection Kit and the DIG Luminescent Detection Kit*.*

Probe synthesis

The PCR DIG Labeling Mix is designed for the synthesis of DIG-labeled hybridization probes with PCR. All types of template DNA can be used for probe synthesis. The amount of labeled PCR product used for hybridization depends on the following parameters:
- Hybridization format
- Targeted sensitivity
- Efficiency of the PCR
- Primer/template pair

Typically, 10 µl of the PCR per 1 ml of hybridization solution yields good results.

⚠ ***When using genomic DNA as a template, side products may be observed. These products are also DIG-labeled during PCR, resulting in a higher background or false-positive signals.***

2.2. Protocols

Standard labeling assay

1. Add the following components to an autoclaved microcentrifuge tube on ice:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade	variable	–
PCR buffer, 10x conc.,15 mM $MgCl_2$ [1]	10	1.5 mM $MgCl_2$
PCR DIG Labeling Mix	10	200 µM dNTPs
Primer 1	1	0.1 – 1 µM
Primer 2	1	0.1 – 1 µM
Taq DNA Polymerase	0.2 – 1	1 – 5 U/100 µl
Template DNA	variable	variable
Total volume	**100 µl**	–

2. Mix reagents thoroughly 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.
   With top heater thermal cyclers, omit the oil overlay.

4. Place samples in the thermal cycler and start PCR.
   When using the PCR DIG Labeling Mix, use the same PCR conditions established for a defined primer/template pair.

5. Store the DIG-labeled PCR probe according to the following table:

Storage	Stability
Short-term	Store at +2 to +8°C until the PCR product is used for hybridization.
Long-term	Store at −15 to −25°C for at least 1 year.

[1] These reagents are supplied with the PCR Buffer Set*.

2.3. Parameters

Working Concentration

Use 10 µl of the PCR DIG Labeling Mix in a standard 100 µl PCR assay.

3. Additional Information on this Product

3.1. Quality Control

The PCR DIG Labeling Mix is function tested in PCR. Amplification products are assayed by dot blot and in hybridization experiments. The PCR DIG Labeling Mix is free of DNases and RNases according to current quality control procedures.

Polymerase chain reaction

Composition and cycling conditions of a standard PCR assay for the synthesis of DIG-labeled PCR products are described in the following table.

Additional information can be found in the documentation for Taq DNA Polymerase.*

Step	Description		
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	Plasmid DNA		1 pg
	PCR primer 1		165 ng
	PCR primer 2		165 ng
	PCR DIG Labeling Mix		10 µl
	10x PCR reaction buffer (15 mM $MgCl_2$)		10 µl
	Taq DNA Polymerase		2.5 U
	Final Volume		**100 µl**
Cycling conditions	1 cycle	Denaturation	7 minutes, +95°C
	30 cycles	Denaturation	45 seconds, +95°C
		Annealing	1 minute, +60°C
		Elongation	2 minutes, +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 to 5 µl of the PCR can be detected.

Hybridization

The plasmid template is spotted in a dilution series on a Nylon Membrane* and hybridized with the DIG-labeled PCR fragment using 10 µl of the PCR 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.

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

Editorial changes.
Layout changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
NBT/BCIP Stock Solution	8 ml	11 681 451 001
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
DIG Luminescent Detection Kit	1 kit, 50 blots of 10 cm x 10 cm	11 363 514 910
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
Taq DNA Polymerase, 5 U/µl	custom fill, 5 U/µl	11 147 633 103
Tth DNA Polymerase	custom fill	11 485 954 103
PCR DIG Probe Synthesis Kit	1 kit, 25 reactions of 50 µl final volume each. One reaction can produce enough labeled probe to analyze 650 cm^2 of blot membrane.	11 636 090 910
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
PCR Buffer Set	1 set, 2 x 1 ml of each solution	11 699 121 001

4.4. Trademarks

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

4.5. License Disclaimer

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

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

To ask questions, solve problems, suggest enhancements or 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.

0621.11583689001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany