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

PCR DIG Labeling Mix^{PLUS}

Version: 08
Content Version: January 2022

For direct labeling of amplification products with DIG-dUTP in PCR and for carryover prevention.

Cat. No. 11 835 289 910 2 x 250 µl
2 x 50 PCR assays of 50 µl final volume each or 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^{PLUS}, 10x conc.	dNTP labeling mixture: Lithium salts of 2 mM dATP, dCTP, dGTP each, 5.7 mM dUTP, 0.3 mM digoxigenin-11-dUTP (DIG-11-dUTP) 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^{PLUS}, 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 control reaction assay

- Water, PCR Grade*
- PCR Buffer Set*
- Uracil-DNA Glycosylase*, heat labile
- Taq DNA Polymerase, 1 U/µl*
- Mineral oil
- PCR ELISA, DIG-Detection* kit
- PCR ELISA, DIG-Labeling* kit

1.4. Application

The PCR DIG Labeling Mix^{PLUS} can be used in several applications:

- When added directly to polymerase chain reactions, the DIG-labeled nucleotide is incorporated into the PCR product.
- The incorporation of dUTP instead of dTTP allows the degradation of contaminating PCR products from previous amplification reactions with Uracil-DNA Glycosylase* (UNG) to prevent carryover contamination.
- The incorporated digoxigenin-label renders the sensitive detection of the PCR product with anti-digoxigenin-conjugates, for example in the PCR ELISA (DIG-Labeling/ DIG-Detection)*.

i *The PCR DIG Labeling Mix^{PLUS} usually gives comparable signal intensities to the PCR DIG Labeling Mix*.*

2. How to Use this Product

2.1. Before you Begin

Mg^{2+} Concentration

Increased dUTP concentration requires an increased concentration of $MgCl_2$ in the PCR buffer. Increase the $MgCl_2$ concentration by 0.5 mM to 1 mM in comparison to the identical PCR assay without dUTP incorporation.
⚠ ***For optimal amplification results, optimization of the $MgCl_2$ concentration is required.***

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.

2.2. Protocols

Control reaction assay

The PCR DIG Labeling Mix^{PLUS} is 10x concentrated. For each reaction, add 10% of the final volume to the reaction mix.

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

Reagent	Control Volume [µl]	Final conc.
Water, PCR Grade	18.5	–
PCR DIG Labeling Mix^{PLUS}	5	200 µM
Control primer mixture	5	250 nM each
Taq DNA Polymerase 1 U/µl	1.5	1.5 U
Uracil-DNA Glycosylase 1 U/µl	2	2 U
PCR buffer, without $MgCl_2$, 10x conc.(1)	5	1x
25 mM $MgCl_2$ Stock Solution(1)	3	1.5 mM $MgCl_2$
Template DNA	10	variable (final 30 ng/50 µl)
Total volume	**50**	

2. Mix reagents thoroughly and centrifuge briefly to collect the sample at the bottom of the tube.

3. Overlay with mineral oil to reduce evaporation of the mix during amplification.
   With top heater thermal cyclers, omit the oil overlay.

4. Incubate samples 10 minutes at +20°C for UNG digestion.

5. Place samples in a thermal cycler.
   – The cycling program for the control reaction including inactivation of the Uracil-DNA Glycosylase and denaturation of the template DNA is given below:

Thermal cycler program		
	Temp. [°C]	Duration [s]
Pre-incubation[2]	95	120
3-Step amplification	No. of cycles: 35	
	95	45
	60	60
	72	120
Last cycle	72	up to 600[3]

❻ Analyze PCR products with the PCR ELISA, DIG-Detection* kit.

(1) These reagents are supplied with the PCR Buffer Set*.
(2) Uracil DNA glycosylase inactivation/ Denaturation of the template
(3) Final elongation to ensure completion of reaction.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

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	
ℹ *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

Product	Pack Size	Cat. No.
Reagents, kits		
PCR DIG Labeling Mix	2 x 250 µl, 2 x 25 PCR assays of 100 µl final volume each	11 585 550 910
Taq DNA Polymerase, 1 U/µl	250 U, 1 U/µl 200 reactions in a final volume of 50 µl	11 647 679 001
	1,000 U, 4 x 250 U 800 reactions in a final volume of 50 µl	11 647 687 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
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
PCR ELISA, DIG-Detection, 5-pack	1 kit, 480 detection reactions, five microplates. The number of tests depends on the number of required sample dilutions and standard and/or control reactions.	11 965 409 910

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 and select the corresponding product catalog.

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

0122.11824899001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany