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

PCR Nucleotide Mix^PLUS^

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Cat. No. 11 888 412 001 2 x 100 µl
200 PCR reactions in 50 µl

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	PCR Nucleotide Mix^{PLUS}	▪ Ready-to-use nucleotide mix. ▪ Clear, colorless solution of the sodium salts of dATP, dCTP, dGTP, each at a concentration of 10 mM, and dUTP at a concentration of 30 mM in water for a total volume of 100 µl, pH 8.3.	2 vials, 100 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 Nucleotide Mix^{PLUS}	Store at −15 to −25°C. ⚠ ***It will withstand 50 freeze/thaw cycles.***

1.3. Additional Equipment and Reagent required

For PCR with Taq Polymerase

- PCR-buffer, 10x conc., without $MgCl_2$*, or supplied with Taq DNA Polymerase 1 U/µl
- $MgCl_2$ Stock Solution, 25 mM*
- Taq DNA Polymerase 1 U/µl*
- Uracil DNA-Glycosylase, heat-labile*
- Water, PCR Grade*

For RT-PCR with Tth DNA Polymerase

- Tth DNA Polymerase 5 U/µl*
- Uracil DNA-Glycosylase, heat-labilel*
- Water, PCR Grade*

1.4. Application

The PCR Nucleotide Mix^{PLUS} is optimized for use in amplification reactions. The incorporation of dUTP in place of dTTP enables the degradation of contaminating PCR products from former reactions with Uracil DNA-Glycosylase (UNG) to prevent carryover contamination from previous amplifications.

2. How to Use this Product

2.1. Before you Begin

General Considerations

The increased dUTP concentrations used in the PCR Nucleotide Mix[PLUS] require a higher $MgCl_2$ concentration, compared with standard PCR using dTTP. For best results, increase the $MgCl_2$ concentration to 0.5 to 1.0 mM above that used for the identical PCR setup without dUTP incorporation.

- To obtain maximal efficiency of amplification, titrate the Mg^{2+} concentration in advance.
- Optimal reaction conditions are dependent on template DNA and primer. In particular, incubation times, temperatures, Mg^{2+}, enzyme, template DNA and primer concentrations should be optimized for each new primer/ template pair.
- dUTP-containing PCR products can be detected using widely used methods. It is also possible to create labeled PCR products by using a 5′-labeled PCR primer, such as labeled with digoxigenin, biotin, or fluorescence.

To enable decontamination of PCR or RT-PCR, dUTP in place of dTTP is incorporated into the PCR product. Subsequent reactions may then be treated with Uracil DNA-Glycosylase (UNG). Avoiding the need to reopen the reaction vial, the vials are incubated at +20°C, resulting in the degradation of potentially contaminating uracil-containing amplification products. During this step, template DNA and RNA remain unaffected, since normal DNA does not contain uracil, and RNA does not serve as a substrate for UNG. Before starting the actual thermocycling program, UNG is inactivated by incubation at +95°C. Uracil DNA-Glycosylase, heat-labile* is particularly useful, as it is fully inactivated already after incubation at +95°C for 2 minutes. The natural enzyme from *E. coli* requires incubating the reaction mixture for 10 minutes at +95°C. The shorter heat treatment substantially reduces the risk for loosing the template nucleic acid, which typically is present at low concentrations only. This is of particular importance, when performing RT-PCR. Use Uracil DNA-Glycosylase, heat-labile* as described in the examples below.

2.2. Protocols

Protocols for decontamination and amplification

For PCR with Taq Polymerase

For a larger number of reactions, prepare a master mix containing water, nucleotides, primer, Taq DNA Polymerase, and UNG.

1. To an autoclaved reaction tube on ice, add the components in the order listed for each 50 µl or 100 µl reactions:

Reagent	Volume [µl]/ 50 µl reaction	Volume [µl]/ 100 µl reaction	Final conc.
Water, PCR Grade*	variable	variable	–
PCR Nucleotide Mix[PLUS]	1	2	200 µM of dATP, dCTP, dGTP, 600 µM dUTP
Forward primer	variable	variable	250 nM
Reverse primer	variable	variable	250 nM
Uracil DNA-Glycosylase, heat-labile 1 U/µl*	2	2	2 U
Taq DNA Polymerase 1 U/µl*	1.5	2.5	–
PCR-buffer, 10x conc., without $MgCl_2$*	5	10	1x
$MgCl_2$ Stock Solution 25 mM*	variable	variable	1 to 3 mM
Template DNA	variable	variable	–
Final Volume	**50**	**100**	

2 Mix and centrifuge briefly.

3 To reduce evaporation, add carefully 100 µl mineral oil to the top of the mixture.
– Mineral oil can be omitted if you are using a PCR cycler that does not require an oil overlay, according to the recommendations of the manufacturer.

4 Place the sample in a thermocycler and start an appropriate cycling program. See example below:

Step	Temperature [°C]	Time	Number of Cycles
UNG digestion	20	2 min	1
UNG inactivation and denaturation of the template	95	2 min	1
Denaturation Annealing Elongation	95 50 – 70 72	45 sec 1 min 2 min	30
Elongation, using prolonged elongation time	72	up to 10 min	1

The annealing temperature depends on the melting temperature for the primers used. Typically, use the same cycle numbers and temperature profiles successfully established in your reaction using dTTP.

5 Keep the samples at +2 to +8°C for short-term storage up to few hours or store them at −15 to −25°C.

For RT-PCR with Tth DNA Polymerase

For a larger number of reactions, prepare a master mix containing water, nucleotides, primer, Tth Polymerase, and UNG.

1 To an autoclaved reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]/50 µl reaction	Final conc.
Water, PCR Grade*	variable	–
PCR Nucleotide Mix^{PLUS}	1.5	300 µM of dATP, dCTP, dGTP, 900 µM dUTP
Forward primer	variable	450 nM
Reverse primer	variable	450 nM
Manganese acetate stock solution 25 mM	5	2.5 mM
Tth Polymerase 5 U/µl*	0.5	2.5 U
Uracil DNA Glycosylase, heat-labile, 1 U/µl*	2	2 U
5x RT-PCR-buffer for Tth Polymerase*	10	1 x
Template RNA	variable	1 ng – 1 µg
Final Volume	**50**	

2 Mix and centrifuge briefly.

3 To reduce evaporation, add carefully 100 µl mineral oil to the top of the mixture.
Mineral oil can be omitted if you are using a PCR cycler that does not require an oil overlay according to the recommendations of the manufacturer.

❹ Place the sample in a thermocycler and start an appropriate cycling program. See example below:

Step	Temperature [°C]	Time	Number of Cycles
UNG digestion	20	2 min	1
UNG inactivation	95	2 min	1
Reverse transcriptase reaction	60 to 70	30 min	1
Denaturation Annealing Elongation	94 50 – 70 72	30 sec 30 sec 45 sec	10
Denaturation Annealing Elongation, plus 5 sec cycle elongation to be added to each cycle	94 50 – 70 72	30 sec 30 sec 45 sec + 5 sec/cycle	20 – 30
Elongation, using prolonged elongation time	72	up to 10 min	1

ⓘ *The annealing temperature depends on the melting temperature for the primers used. Typically, use the same cycle numbers and temperature profiles successfully established in your reaction using dTTP.*

❺ Keep the samples at +2 to +8°C for short-term storage up to a few hours or store at −15 to −25°C.

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	
ⓘ *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.**	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ 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 Buffer	3 x 1 ml, 10x conc	11 699 105 001
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
Tth DNA Polymerase	500 U, 2 x 250 U, 5 U/µl	11 480 022 001
$MgCl_2$ Stock Solution	3 x 1 ml	11 699 113 001

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.

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