For general laboratory use. Not for use in diagnostic procedures FOR *IN VITRO* USE ONLY.

PCR Nucleotide Mix^{PLUS}

Premixed deoxynucleotide solution for use in „PCR and RT-PCR carry-over prevention“. Sufficient for 200 PCR-reactions at a volume of 50 µl, or for 100 PCR-reactions at a volume of 100 µl or for 133 RT-PCR-reactions at a volume of 50 µl.

Cat. No. 11 888 412 001

2 × 100 µl

Version March 2006

Store at −15 to −25°C

Product description

PCR Nucleotide Mix^{PLUS} is a 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 PCR grade water. This nucleotide mixture can be added directly to polymerase chain reactions.

The incorporation of dUTP in place of dTTP allows the degradation of contaminating PCR products from former reactions with uracil-DNA glycosylase (UNG) to prevent carry over contamination from previous amplifications.

Storage and stability

The PCR Nucleotide Mix^{PLUS} is stable through the control date printed on the vial when stored at −15 to −25°C and will withstand 50 freeze/thaw cycles.

Application

To allow 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 of re-opening 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 Glycosilase, heat-labile* is particularily 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. We therefore recommend the use of Uracil DNA Glycosilase, as described in the examples below.

Instructions for use

General Remarks

Increased dUTP concentrations, as used in the PCR Nucleotide Mix^{PLUS} require a higher concentration of $MgCl_2$ in the PCR buffer, when compared with standard PCR, using dTTP. We therefore recommend to increase the $MgCl_2$ concentration to a concentration, 0.5 mM to 1.0 mM above that, used for the identical PCR setup without dUTP incorporation. For obtaining 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 and temperatures, concentration of Mg^{2+} and enzyme but also concentration of template-DNA and primer should be optimised for best results for each new primer/template pair.

dU-containing PCR products can be detected with the commonly used methods. It is also possible to create labeled PCR products by using a 5' labeled PCR primer (e. g. labeled with Digoxigenin, Biotin or Fluorescence).

Exemplary protocols for decontamination and amplification

A. Example for PCR with Taq-polymerase

Additionally required reagents

- PCR-buffer, 10 × conc., without $MgCl_2$, 3 × 1 ml*, or supplied with Taq DNA polymerase 1 unit/µl
- Taq DNA polymerase 1 unit/µl*
- Uracil DNA glycosylase, heat-labile*
- Water, PCR grade, autoclaved

1. To prepare a reaction mixture for amplification add the following reagents in the same order as described in the table below. Keep the tubes on ice during pipetting. For a larger number of reactions we recommend to prepare a master mix containing water, nucleotides, primer, Taq-DNA-polymerase and UNG

Reagent	Volume/ 50µl reaction	Volume/ 100µl reaction	Final concentration
Water, PCR grade	variable	variable	
PCR Nucleotide Mix ^{PLUS}	1µl	2µl	200 µM dATP, dCTP, dGTP, 600 µM dUTP
Upstream primer	variable	variable	250 nM
Downstream primer	variable	variable	250 nM
Uracil DNA glycosylase, heat-labile 1 unit/µl*	2µl	2µl	2 units
Taq DNA polymerase, 1 unit/µl*	1.5µl	2.5µl	
PCR-buffer, 10 × conc., without $MgCl_2$*	5µl	10µl	1 ×
$MgCl_2$-stock solution, 25mM *	variable	variable	1-3 mM
Template DNA	variable	variable	
Final volume	**50 µl**	**100 µl**	

* supplied with Taq DNA polymerase 1 unit/µl

2. Vortex the mixture and centrifuge briefly to collect the reagents at the bottom of the tube.

3. Carefully add 100 ml mineral oil to the top of the mixture to reduce evaporation.
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. An example is given below:

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Cycle(s)	Time	Temperature	Purpose
1 ×	2 min.	20°C	UNG-digestion
1 ×	2 min.	95°C	UNG-inactivation and denaturation of the template
30 ×	45 s 1 min 2 min	95°C 50-70°C 72°C	Denaturation Annealing Elongation
1 ×	up to 10 min	72°C	Elongation, using prolonged elongation time

Note: 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 samples at 4°C for short-term storage (up to a few hours) or store samples frozen at −15 to −25°C for prolonged storage.

B. Example for RT-PCR with Tth DNA polymerase

Additionally required reagents

- Tth DNA polymerase 5 U/µl*
- Uracil DNA glycosylase, heat-labile 1 U/µl*
- Water, RT-PCR grade, autoclaved, and Velcorin[1)]-treated

1. To prepare a reaction mixture for amplification add the following reagents in the same order as described in the table below. Keep the tubes on ice during pipetting. For a larger number of reactions we recommend to prepare a master mix containing water, nucleotides, primer, Tth-polymerase and UNG.

Reagent	Volume/ 50 µl reaction	Final Concentration
Water, RT-PCR grade	variable	
PCR Nucleotide Mix PLUS	1.5 µl	300 µM dATP, dCTP, dGTP, 900 µM dUTP
Upstream primer	variable	450 nM
Downstream primer	variable	450 nM
Manganese acetate stock solution, 25 mM*	5 µl	2.5 mM
Tth DNA Polymerase, 5 units/µl*	0.5 µl	2.5 units
Uracil DNA glycosylase, heat-labile, 1 unit/µl*	2 µl	2 units
5 × RT-PCR buffer for Tth DNA Polymerase*	10 µl	1 ×
Template RNA	variable	1 ng-1 µg
Final volume	**50 µl**	

* supplied with Tth DNA Polymerase

2. Vortex the mixture and centrifuge briefly to collect the reagents at the bottom of the tube.
3. Carefully add 100 ml mineral oil to the top of the mixture to reduce evaporation.
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. An example is given below:

Cycle(s)	Time	Temperature	Purpose
1 ×	2 min	20°C	UNG-digestion
1 ×	2 min	95°C	UNG-inactivation
	30 min	60-70°C	Reverse transcriptase reaction
10 ×	30 s 30 s 45 s	94°C 50-70°C 72°C	Denaturation Annealing Elongation
20-30 ×	30 s 30 s 45 s + 5 s/cycle	94°C 50-70°C 72°C	Denaturation Annealing Elongation plus 5 s cycle elongation to be added to each cycle
1 ×	up to 10 min	72°C	Elongation, using prolonged elongation time

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

5. Keep samples at 4°C for short-term storage (up to a few hours) or store samples frozen at −15 to −25°C for prolonged storage.

Quality Control

Function testing in DNA amplification reaction

Each lot of PCR Nucleotide Mix^{PLUS} is function-tested to ensure specific DNA amplification. With primers specific for the β-globin gene, 50 ng of human placental DNA is amplified according to the procedure detailed in the "Instructions for Use" section. Ten microliters of the amplification product is then subjected to electrophoresis in an agarose gel and stained with ethidium bromide. The 1.5 kb band predicted by the template and primers is clearly visible, indicating specific amplification.

Absence of contaminating deoxyribo-nucleases

Each lot of PCR Nucleotide Mix^{PLUS} is tested to ensure the absence of deoxyribonucleases (DNases) by incubating it for 4 hours at 37°C with 0.5 µg of the supercoiled plasmid pBR328. The sample is then subjected to electrophoresis in an agarose gel and stained with ethidium bromide. No degradation products corresponding to a decrease in the supercoiled form (and to an increase in relaxed or linearized DNA) are observed, indicating the absence of contaminating RNases.

Absence of contaminating ribonucleases

Each lot is tested to ensure the absence of ribonucleases (RNases) by incubating it for 6 hours at 37°C with 2.16 µg MS2 RNA. The sample is then subjected to electrophoresis in a formaldehyde agarose gel and stained with ethidium bromide. No degradation products are observed, indicating the absence of contaminating RNases.

NOTICE TO PURCHASER:

DISCLAIMER OF LICENSE

This product is optimized for use in the polymerase chain reaction (PCR) covered by patents owned by F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR Process for certain research and development activities accompanies the purchase of certain Roche, Applied Biosystems or other licensed suppliers' reagents when used in conjunction with an authorized thermal cycler, or is available from Applied Biosystems. Diagnostic purposes require a license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

* available from Roche Applied Science

[1)] Velcorin is a trademark of Bayer AG, Leverkusen,Germany

Available Printed Material:

PCR Product Family Flyer

Lab FAQS „Find a Quick Solution“

Molecular Weight Markers for Nucl. Acids

PCR Grade Nucleotides

Restriction Enzyme Poster

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 and for information concerning quantitative realtime PCR the LightCycler homepage at: http://roche-applied-science.com/lightcycler/

Product	Pack Size	Cat. No.
Set of Deoxy-Nucleotides, PCR Grade	4× 25 µmol (4× 250 µl)	11 969 064 001
	4× 125 µmol (4× 1250 µl)	03 622 614 001
Uracil-DNA Glycosylase, heat labile	100 units 500 units (500 µl)	11 775 367 001 11 775 375 001
PCR Nucleotide Mix	100 reactions 10 × 200 µl (1000 reactions)	11 581 295 001 11 814 362 001
dATP, PCR Grade	25 µmol 125 µmol,	11 934 511 001 11 969 013 001
dCTP, PCR Grade	25 µmol 125 µmol,	11 934 520 001 11 969 021 001
dGTP, PCR Grade	25 µmol 125 µmol,	11 934 538 001 11 969 030 001
dTTP, PCR Grade	25 µmol 125 µmol,	11 934 546 001 11 969 048 001
dUTP, PCR Grade	25 µmol 125 µmol,	11 934 554 001 11 969 056 001
PCR Buffer Set	2 × 1 ml of both buffers (2 × 2 ml)	11 699 121 001
PCR Buffer without $MgCl_2$, (10 ×)	3 × 1 ml	11 699 105 001
Thin-walled PCR Tubes	1000 tubes (200 µl) 1000 tubes (500 µl),	11 667 041 001 11 667 050 001
GC RICH PCR System	1 kit	12 140 306 001
High Fidelity PCR Master	1 kit	12 140 314 001
Expand High Fidelity PCR System	100 units 500 units (2 × 250 units) 2500 units (10 × 250 units)	11 732 641 001 11 732 650 001 11 759 078 001
Expand Long Template PCR System	100 units 500 units 2500 units (10 × 250 units)	11 681 834 001 11 681 842 001 11 759 060 001

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 Pack Inserts and Material Safety Data Sheets.

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