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

PCR Nucleotide Mix

Version: 10
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Premixed solution of highly pure PCR-grade deoxynucleotides (dATP, dGTP, dCTP, and dTTP, 10 mM each).

Cat. No. 11 581 295 001	200 µl 500 reactions of 20 µl final reaction volume
Cat. No. 04 638 956 001	5 x 200 µl 2,500 reactions of 20 µl final reaction volume.
Cat. No. 11 814 362 001	10 x 200 µl 5,000 reactions of 20 µl final reaction volume.

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	PCR Nucleotide Mix	Clear, colorless solution of the sodium salts of dATP, dCTP, dGTP, and dTTP, each at a concentration of 10 mM in water, pH 8.3.	11 581 295 001	1 vial, 200 µl
			04 638 956 001[1]	5 vials, 200 µl each
			11 814 362 001	10 vials, 200 µl each

[1] Available through US Consignment Freezer Programs only.

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	Store at −15 to −25°C. ⚠ ***It will withstand 50 freeze/thaw cycles.***

1.3. Application

The PCR Nucleotide Mix is optimized for use in amplification reactions. This ready-to-use mix can be added directly to all types of amplification reactions and other primer-extension reactions, such as:

- PCR
- One-step RT-PCR
- cDNA synthesis reactions

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as genomic or plasmid DNA, cDNA suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).
- Use 10 to 500 ng complex genomic DNA or 0.1 to 10 ng plasmid DNA/cDNA. A good starting concentration is 250 ng genomic DNA or 1 ng plasmid DNA.

⚠ ***Store the template DNA in either Water, PCR Grade* or 5 to 10 mM Tris-HCl, pH 7 to 8. Avoid dissolving the template in TE buffer since EDTA chelates Mg^{2}+.***

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg^{2}+ vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg^{2}+ concentration and the amount of enzyme used per assay to ensure optimal efficiency of DNA synthesis. As a starting point, use the following guidelines:

- Thaw the solution, vortex, and centrifuge shortly before beginning the procedure.
- Optimal enzyme concentration: 0.5 to 2.5 U/50 µl. A concentration of 1.25 U/50 µl will usually produce satisfactory results.
- Optimal Mg^{2}+ concentration can vary between 1.5 mM and 5 mM. In most cases, a Mg^{2}+ concentration of 1.5 mM will produce satisfactory results if you use 200 µM of each dNTP.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. If you increase the dNTP concentration, you must also increase the Mg^{2}+ concentration.

2.2. Protocols

Preparation of PCR master mixes

Prepare two PCR master mixes. Master Mix 2 contains enzyme and reaction buffer; Master Mix 1 contains all other reaction components. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template during the reaction setup. If you are setting up multiple reactions, the volume of each master mix should be 110% of the volume needed for all the samples. For example, to prepare Master Mix 2 for 10 reactions, make 275 µl of the mix. The extra volume allows for losses during pipetting.

Preparation of master mix 1

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

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2. Prepare a 10x-concentrated solution of each respective primer.
   ℹ *If you are using, for example, the final concentration of 0.5 µM for each primer, the 10x-concentrated solution would contain a 5 µM concentration of the respective primer.*

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3 To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to a final volume of 25	–
PCR Nucleotide Mix (10 mM of each dNTP)	1	200 µM of each dNTP
Forward primer 1	5	0.1 – 0.6 µM
Reverse primer 2	5	0.1 – 0.6 µM
Template DNA	variable	0.1 – 500 ng (Genomic DNA: 10 ng to 500 ng Plasmid DNA: 0.1 ng to 15 ng)
Final Volume	**25**	

4 Mix and centrifuge briefly.

Preparation of master mix 2

1 Thaw the reagents and store on ice.
– Briefly vortex and centrifuge all reagents before setting up the reactions.

2 To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	19.75	–
PCR reaction buffer, 10x	5	1x (1.5 mM $MgCl_2$)
Taq DNA Polymerase* (5 U/µl)	0.25	1.25 U/reaction
Final Volume	**25**	

3 Mix and centrifuge briefly.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

1 For each reaction, combine 25 µl Master Mix 1 and 25 µl Master Mix 2 in a thin-walled PCR tube on ice.
– Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
Carefully overlay the reaction with mineral oil if required by your thermal cycler.

⚠ ***Start thermal cycling immediately. Do not store the combined reaction mix on ice.***

2 Place your samples in a thermal block cycler and use either of the thermal profiles below to perform PCR.

Thermal Profile A has a fixed extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 95[1]	2 min	1
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72[3]	15 – 30 sec 30 – 60 sec 45 sec – 3 min	25 – 30
Final Elongation	72[3]	7 min	1
Cooling	4	indefinitely	

Thermal Profile B has a gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 95[1]	2 min	1
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72[3]	15 – 30 sec 30 – 60 sec 45 sec – 3 min	10
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72[3]	15 – 30 sec 30 sec 45 sec – 3 min + 5 sec cycle elongation for each successive cycle[4]	15 – 20
Final Elongation	72[3]	7 min	1
Cooling	4	indefinitely	

3 After cycling, use samples immediately or store them at −15 to −25°C for later use.

For best results, check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker. In addition, purify the PCR product with the High Pure PCR Product Purification Kit, for example, before performing nested PCR.

[1] The denaturation temperature can vary between +92 and +95°C. The standard denaturation temperature is +94°C.
[2] Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
[3] For PCR products up to 1 kb, elongation temperature should be approximately +72°C; for PCR products >1 kb, elongation temperature should be approximately +68°C.
[4] For example, cycle number 11 is 5 seconds longer than cycle 10. Cycle number 12 is 10 seconds longer than cycle 10. Cycle number 13 is 15 seconds longer than cycle 10, etc.

2.3. Parameters

pH Stability

Since most amplification reactions are performed between pH 8 and 9, PCR-Grade deoxynucleotides are more resistant to chemical stress caused by pH shifts, compared to deoxynucleotides stored at pH 7 to 7.5.

Purity

Purified to the highest possible purity with high-resolution HPLC (>99% dNTP, <0.9% dNDP). No additives or stabilizers are added.
Special manufacturing process synthesizes high quality PCR Grade deoxynucleotides that are virtually free of all contaminants (including dNTPs with modified bases, tetra- and pyrophosphates) that inhibit amplification reactions.

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.	
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		
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
Taq DNA Polymerase, 5 U/µl	100 U, 5 U/µl, 80 reactions	11 146 165 001
	500 U, 5 U/µl, 400 reactions	11 146 173 001
	4 x 250 U, 5 U/µl, 800 reactions	11 418 432 001
	10 x 250 U, 5 U/µl, 2,000 reactions	11 596 594 001
	20 x 250 U, 5 U/µl, 4,000 reactions	11 435 094 001

4.4. Trademarks

MAGNA PURE is a trademark of Roche.
All other 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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