

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



# Deoxynucleoside Triphosphate Set PCR Grade

 **Version: 09**  
Content Version: April 2021

Sodium salt solution

<b>Cat. No. 11 969 064 001</b>	4 x 250 µl 4 x 25 µmol, 100 mM
<b>Cat. No. 03 622 614 001</b>	4 x 1,250 µl 4 x 125 µmol, 100 mM

**Store the set at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Deoxynucleoside Triphosphate Set, PCR Grade, dATP	100 mM clear, colorless, sodium salt solutions in water, pH 8.3.	11 969 064 001	4 vials, 250 µl each
			03 622 614 001	4 vials, 1,250 µl each
2	Deoxynucleoside Triphosphate Set, PCR Grade, dCTP		11 969 064 001	4 vials, 250 µl each
			03 622 614 001	4 vials, 1,250 µl each
3	Deoxynucleoside Triphosphate Set, PCR Grade, dGTP		11 969 064 001	4 vials, 250 µl each
			03 622 614 001	4 vials, 1,250 µl each
4	Deoxynucleoside Triphosphate Set, PCR Grade, dTTP		11 969 064 001	4 vials, 250 µl each
			03 622 614 001	4 vials, 1,250 µl each

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at  $-15$  to  $-25^{\circ}\text{C}$ , the set is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	dATP	Store at $-15$ to $-25^{\circ}\text{C}$ .
2	dCTP	
3	dGTP	
4	dTTP	

### 1.3. Additional Equipment and Reagent required

#### Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

#### For PCR

- PCR primers
- PCR reaction buffer, 10x conc.
- Template DNA
- Water, PCR Grade\*
- Taq DNA Polymerase\*

### 1.4. Application

The Deoxynucleoside Triphosphate Set, PCR Grade can be used for:

- Reverse transcription
- PCR
- RT-PCR
- DNA labeling reactions
- Sequencing/cycle sequencing

**i** See the protocols in the Instructions for Use of the appropriate products.

## 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 ng to 250 ng complex genomic DNA or 0.1 ng to 15 ng plasmid DNA/cDNA.

**⚠ 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<sup>2+</sup>.**

#### General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg<sup>2+</sup> vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg<sup>2+</sup> concentration and the amount of enzyme used per assay to ensure optimal efficiency of DNA synthesis.

As a starting point, use the following guidelines:

- 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<sup>2+</sup> concentration can vary between 1.5 mM and 5 mM. In most cases, a Mg<sup>2+</sup> 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<sup>2+</sup> concentration.

#### Working Solution

##### Deoxynucleotide mix

For best results, prepare a deoxynucleotide mix containing dATP, dCTP, dGTP, dTTP (10 mM, each). For example, for the preparation of 100 µl nucleotide mix, add 10 µl of dATP, dCTP, dGTP, and dTTP to 60 µl Water, PCR Grade\*.

## 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. The extra volume allows for losses during pipetting.

#### Preparation of master mix 1

*i* See section, **Working Solution** for information on preparing solutions.

- 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*	add up to a final volume of 25	–
dNTP mix (10 mM of each dNTP)	1	200 µM of each dNTP
Forward primer 1	variable	0.1 – 0.6 µM
Reverse primer 2	variable	0.1 – 0.6 µM
Template DNA	variable	0.1 – 250 ng (Genomic DNA: 10 ng to 250 ng Plasmid DNA: 0.1 ng to 15 ng)
<b>Final Volume</b>	<b>25</b>	

- 3 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 <sub>2</sub> )
Taq DNA Polymerase* (5 U/µl)	0.25	1.25 U/reaction
<b>Final Volume</b>	<b>25</b>	

- 3 Mix and centrifuge briefly.

### PCR protocol

*i* 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.

*i* 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.  
*i Thermal Profile A has a fixed extension time.*

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94 <sup>(1)</sup>	2 min	1
Denaturation	94 <sup>(1)</sup>	15 – 30 sec	25 – 30
Annealing	55 – 65 <sup>(2)</sup>	30 – 60 sec	
Elongation	72 <sup>(3)</sup>	45 sec – 3 min	
Final Elongation	72 <sup>(3)</sup>	7 min	1
Cooling	4	indefinitely	

*i 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	94 <sup>(1)</sup>	2 min	1
Denaturation	94 <sup>(1)</sup>	15 – 30 sec	10
Annealing	55 – 65 <sup>(2)</sup>	30 – 60 sec	
Elongation	72 <sup>(3)</sup>	45 sec – 3 min	
Denaturation	94 <sup>(1)</sup>	15 – 30 sec	15 – 20
Annealing	50 – 65 <sup>(2)</sup>	30 sec	
Elongation	72 <sup>(3)</sup>	45 sec – 3 min + 5 sec cycle elongation for each successive cycle <sup>(4)</sup>	
Final Elongation	72 <sup>(3)</sup>	7 min	1
Cooling	4	indefinitely	

- 3 After cycling, use samples immediately or store them at –15 to –25°C for later use.  
*i 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.*

<sup>(1)</sup> The denaturation temperature can vary between +92 and +95°C. The standard denaturation temperature is +94°C.

<sup>(2)</sup> Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.

<sup>(3)</sup> 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.

<sup>(4)</sup> 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

### Purity

PCR-Grade nucleotides contain at least 99% of the relevant deoxynucleoside-triphosphate (dNTP) and less than 0.9% deoxynucleoside-diphosphate (dNDP).

## 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.**

   etc. Stages in a process that usually occur in the order listed.

   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. Supplementary Information

### 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.

