For general laboratory use.

dCTP
PCR Grade, sodium salt

Version: 09
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Cat. No. 11 934 520 001	250 µl 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.
Cat. No. 11 969 021 001	1,250 µl 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.
Cat. No. 03 732 690 001	4 x 1,250 µl 4 x 125 µmol, 100 mM 125,000 standard PCR assays of 20 µl each.

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	dCTP, PCR Grade, Na-salt	▪ Deoxynucleotide of high purity, specially manufactured and tested for PCR and RT-PCR. ▪ Clear, colorless, 100 mM dCTP salt solution in water, pH 8.3.	11 934 520 001	1 vial, 25 µmol (250 µl)
			11 969 021 001	1 vial, 125 µmol (1,250 µl)
			03 732 690 001	4 vials, 125 µmol (1,250 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	dCTP, PCR Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved 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

- Gene-specific PCR primer pair
- Template DNA
- Water, PCR Grade*
- Taq DNA Polymerase*
- dNTPs, PCR Grade or
- PCR Grade Nucleotide Mix*
- PCR buffer, 10x conc. with $MgCl_2$

1.4. Application

Use dCTP, PCR Grade in:

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

2. How to Use this Product

2.1. Before you Begin

General Considerations

The optimal conditions, including times and temperatures, concentration of enzyme, template DNA, and Mg^{2+} vary from system to system and must be determined for each 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:

- Optimal enzyme concentration: 0.5 to 2.5 U/50 µl. The recommended starting concentration is 1.25 U/50 µl.
- Optimal Mg^{2+} concentration can vary from 1.5 mM to 5 mM. In most cases, a Mg^{2+} concentration of 1.5 mM will produce satisfactory results when using 200 µM dNTP (each).
- 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. Increase concentrations of Mg^{2+} when increasing the concentration of dNTP.
- Template concentration, such as human genomic DNA: 10 to 250 ng; plasmid DNA: 0.1 to 15 ng.
- The optimal buffer for the template DNA is either Water, PCR Grade* or 5 to 10 mM Tris, pH 7 to 8.

⚠ ***Avoid dissolving the template in TE buffer since EDTA chelates Mg^{2+}.***

Working Solution

Deoxynucleotide mix (dNTP)

Prepare a mix containing 10 mM each dATP, dCTP, dGTP, dTTP.
For example, for the preparation of 100 µl 10 mM dNTP mix, add 10 µl each of dATP, dCTP, dGTP, dTTP to 60 µl Water, PCR Grade*.

Reactions per pack size

Amount: 25 µmol; 250 µl

A: Reactions/pack size
B: dNTP added per reaction [µl]

Final concentration dNTP in PCR [mM]	PCR Mix Volume					
	25 µl		50 µl		100 µl	
	A	B	A	B	A	B
0.2	5,000	0.050	2,500	0.10	1,250	0.2
0.4	2,500	0.100	1,250	0.20	625	0.4
0.5	2,000	0.125	1,000	0.25	500	0.5
1	1,000	0.250	500	0.50	250	1.0

Amount: 125 µmol; 1,250 µl

A: Reactions/pack size
B: dNTP added per reaction [µl]

Final concentration dNTP in PCR [mM]	PCR Mix Volume					
	25 µl		50 µl		100 µl	
	A	B	A	B	A	B
0.2	25,000	0.050	12,500	0.10	6,250	0.2
0.4	12,500	0.100	6,250	0.20	3,125	0.4
0.5	10,000	0.125	5,000	0.25	2,500	0.5
1	5,000	0.250	2,500	0.50	1,250	1.0

Amount: 4 × 125 µmol, 4 × 1,250 µl

A: Reactions/pack size
B: dNTP added per reaction [µl]

Final concentration dNTP in PCR [mM]	PCR Mix Volume					
	25 µl		50 µl		100 µl	
	A	B	A	B	A	B
0.2	100,000	0.050	50,000	0.10	25,000	0.2
0.4	50,000	0.100	25,000	0.20	12,500	0.4
0.5	40,000	0.125	20,000	0.25	10,000	0.5
1	20,000	0.250	10,000	0.50	5,000	1.0

2.2. Protocols

Preparation of PCR master mixes

For a larger number of reactions, prepare two reaction mixes. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template during the reaction setup. Prepare also a Master Mix for setting up multiple reactions. The Master Mix typically contains all of the components needed for all PCR tests to be performed at a volume 10% greater than that required for the total number of PCR assays.

Preparation of master mix 1

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

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

2. Prepare a 10x-concentrated solution of each respective primer.
   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.

3. To a autoclaved 1.5 ml reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	to make a final volume of 25 µl	–
dNTP Mix* (10 mM)	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 to 250 ng (Genomic DNA: 10 – 250 ng) (Plasmid DNA: 0.1 – 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 autoclaved 1.5 ml 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 conc.	5	1x (1.5 mM $MgCl_2$)
Taq DNA Polymerase, (5U/µl)*	0.25	1.25 U/reaction
Final Volume	**25**	

3. Mix and centrifuge briefly.

PCR

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.
   ⚠ ***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.

3. **Thermal profile A**: Fixed extension time

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94[1]	2 min	1
Denaturation Annealing Elongation	94[1] 50 – 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: Gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94[1]	2 min	1
Denaturation Annealing Elongation	94[1] 50 – 65[2] 72[3]	15 – 30 sec 30 – 60 sec 45 sec – 3 min	10
Denaturation Annealing Elongation	94[1] 50 – 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	

4 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 from +92°C to +95°C. +94°C is the standard denaturation temperature.
[2] Optimal annealing temperature depends on the melting temperature of the primers and on the system used.
[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, etc.

2.3. Parameters

Purity

High purity deoxynucleotide, specially manufactured and tested for PCR and RT-PCR.
dCTP (HPLC), area >99%
dCDP (HPLC), area <0.9%

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		
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
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 Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001

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 general laboratory use.

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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