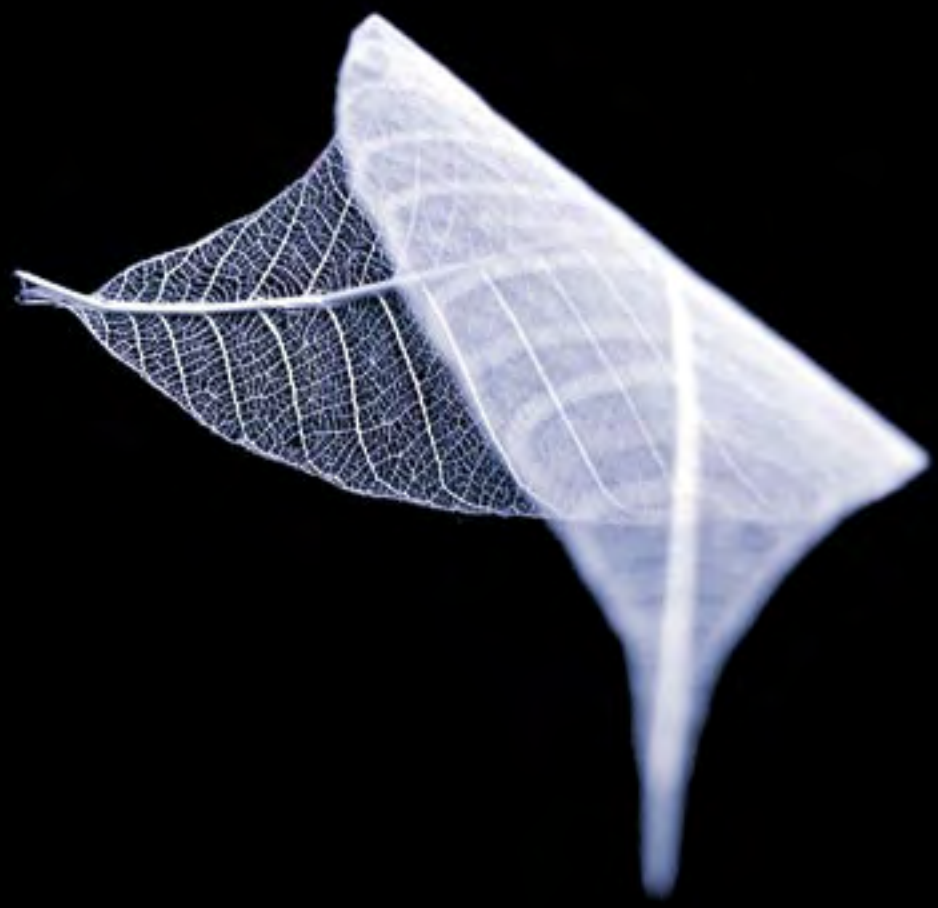
2nd Edition



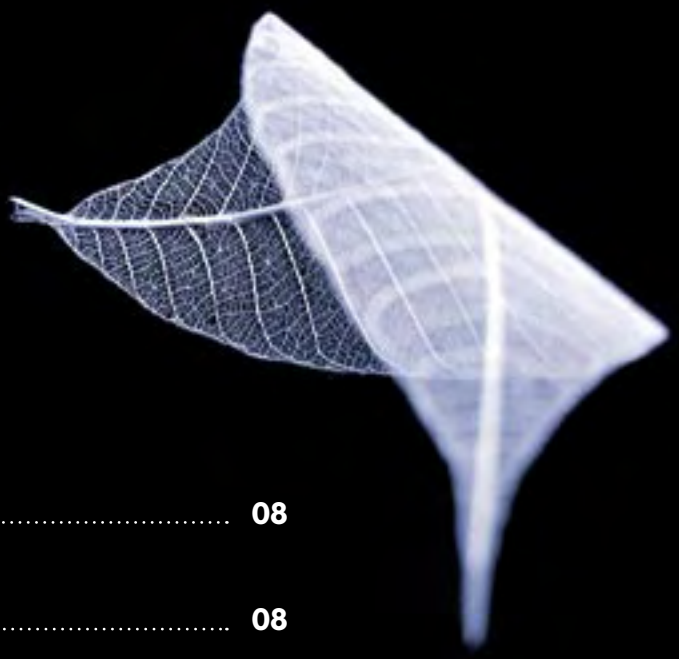
The Definitive Guide to **dNTPs**

Essentials for Successful PCR Reactions

BIOLINE

The Definitive Guide to **dNTPs**

dNTPs Questions & Answers

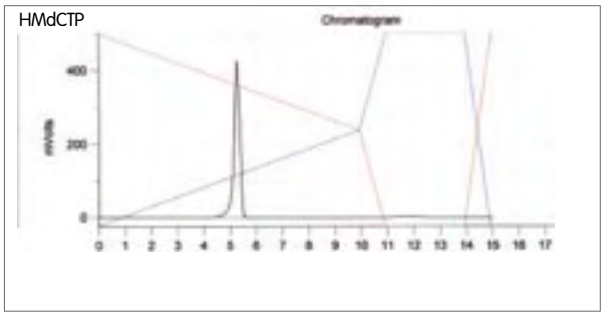


Committed to Science & Product innovation

dNTPs directly from the manufacturer

Since its foundation in 1992, Bioline has been actively involved in the development and manufacture of ultra-pure deoxynucleotides (dNTPs). The state-of-the-art enzymatic manufacturing processes and facilities, combined with stringent quality assurance and control systems, enable us to manufacture dNTPs with the highest level of quality and performance, crucial for sensitive techniques and critical applications in molecular biology.

Bioline is constantly developing and enhancing its production capacity and expertise in the nucleotide area. The most recent development from our laboratories in this field is hydroxymethyl dCTP (HMdCTP). This new development is a novel nucleotide analogue that can be used as a substrate for DNA polymerases. Bioline HMdCTP is manufactured by using a unique method of enzymatic synthesis and possess at least 99% purity by HPLC. HMdCTP can be used to generate PCR products in which cytosines are uniformly replaced by hydroxymethylated cytosines and have exciting applications in forensic DNA analysis, site-directed mutagenesis and in the production of DNA fragments resistant to cleavage by methyl–sensitive endonucleases.



Quality Control

Our purpose built nucleotide manufacturing facilities are ISO 9001:2000 accredited. We follow stringent quality control procedures and rigorous quality assurance systems to guarantee that our dNTPs are of the highest quality.

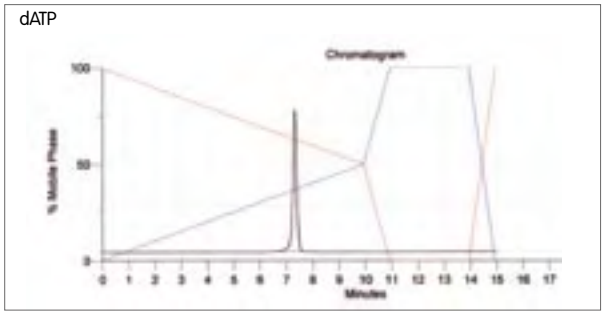
ISO 9001:2000 Certification

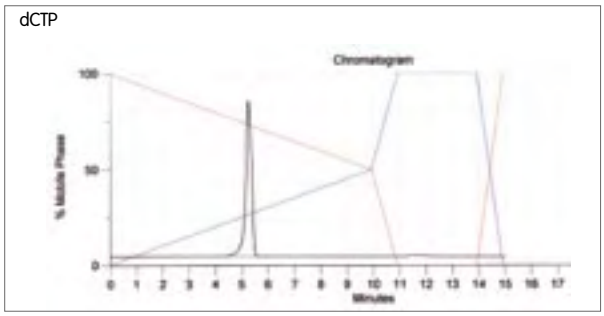
Validation Support Documentation
Rigorous QA / QC Systems
Retention Samples
Product Traceability
Customer On-Site Audits
Regular Inspections

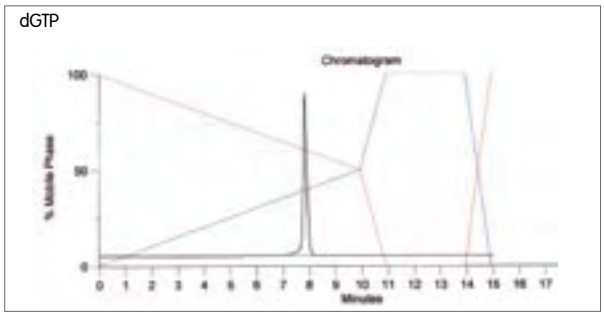
DQS Certified according to
DIN EN ISO 9001:2000
Reg No. 318907 QM
Reg No. 331786 QM

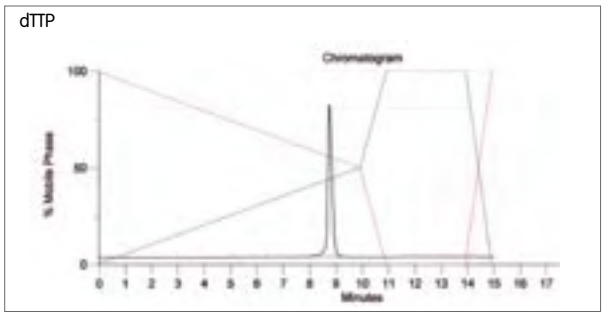
Purity

Ultra-pure dNTPs are enzymatically synthesised from premium-quality dNMPs by phosphorylation, using highly specific production systems in our purpose-built facilities. This manufacturing process eliminates impurities and PCR-specific inhibitors commonly observed in other commercially available dNTP products, such as modified nucleotides, nucleoside tetraphosphates and inorganic pyrophosphates. Bioline dNTPs are purified with quantitative HPLC and possess at least 99% purity.









dNTPs Questions & Answers

Q1. Why are dNTPs important?

dNTPs or deoxynucleoside triphosphates are the “building blocks” for DNA. Two of the essential requirements for achieving a successful PCR are the purity and stability of dNTPs. The use of a highly purified dNTP preparation is particularly recommended for sensitive techniques such as long-range PCR, RT-PCR, multiplex, mutagenesis experiments and real-time applications. The purity of dNTPs is also important when the starting amount of template is minimal.

Q2. How are Bioline dNTPs supplied?

dNTPs can be supplied as either a set or a mix. The mix is presented in a single tube containing a premixed solution of dATP, dCTP, dGTP and dTTP. This solution is ready for use and is optimised for PCR and other applications. The dNTP set contains four separate tubes, one for each deoxynucleotide. dNTPs can be supplied in lyophilised form or dissolved in either lithium or sodium salts.

Q3. What concentration of dNTPs should I use in PCR?

The standard concentration of each dNTP in a PCR reaction is 0.2mM. If the starting stock is a 100mM solution of each dNTP, you need to add 0.1µl of each nucleotide to a 50µl standard PCR reaction. Since this is not convenient, it is recommended to prepare mixes: If the 100mM dNTP stock solutions are mixed in equimolar amounts, the concentration of the mix will be 100mM total or 25mM of each nucleotide. From a 100x stock, you need to add 0.5µl to a 50µl reaction. Bioline offers also more diluted mixes of 40mM total (10mM each), which is a 50x stock solution and 10mM total (2.5mM each) 10x working stock. Several different dNTP Mix formats are available from Bioline as ready-to-go solutions.

Q4. How can I prepare a 50mM dNTP mix from 100mM stocks of individual dNTPs?

Easy. To prepare a 200µl stock solution of 50mM dNTP mix, pipette 25µl of dATP, dCTP, dGTP and dTTP (from 100mM solutions) and add 100µl of ultra-pure water. If other volumes are required, adjust the components up or down accordingly.

Q5. Is it better to use a pre-dispensed dNTP mix rather than a set?

Yes. A factory pre-dispensed and certified dNTP mix preparation offers added convenience by minimising pipetting steps and errors, which could lead to concentration imbalances. And it can be added directly to amplification reactions. Using a dNTP mix ensures reproducibility in your experiments.

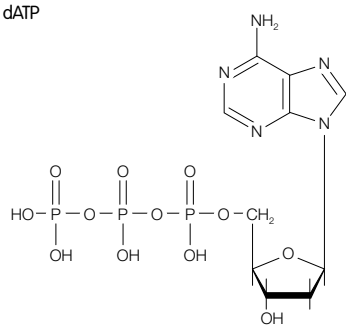
Q6. Which PCR inhibitors can be present in a dNTP preparation that is not ultra-pure?

Your PCR assay can be dramatically affected by a dNTP preparation containing inhibitors, which have resulted from an inadequate manufacturing process. Several parameters must be taken into account when purity is sought, and each dNTP should preferably be free of ribonucleoside triphosphates, other dNTPs, modified nucleotides (methylated, deaminated etc), deoxynucleoside di- and monophosphates (dNDPs and dNMPs), heavy transition metals, inorganic pyrophosphates (PPi) and nucleoside tetraphosphates.

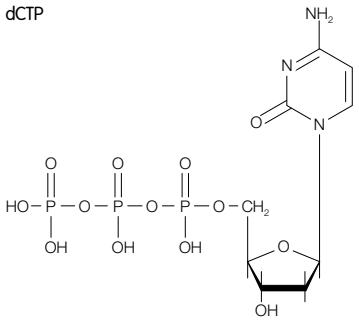
Q7. Can I increase the DNA yield in my PCR by adding dNTPs of higher concentration?

It depends, you probably can increase the DNA yield but you will have to optimise the complete PCR reaction, adjust the buffer, the Mg^{2+} and so on. It is not a matter pertaining only to nucleotides.

dATP



dCTP



dNTPs Questions & Answers

Q8. Does dNTP quality affect fidelity?

Yes. *Taq* polymerase does not discriminate between correct and modified nucleotides, so point-mutations may occur. When proof-reading DNA polymerases are used, this problem is only partially eliminated since the presence of methylated / deaminated nucleotides often blocks DNA synthesis.

Q9. Does dNTP quality affect processivity?

Yes. The quality of dNTPs is especially important for sophisticated reactions such as amplification of long products and real-time PCR. Methylated and deaminated nucleotides exhibit inferior results with proof-reading DNA Polymerases.

Q10. Which test is the most stringent quality criterion for dNTPs?

There is consensus that the most stringent tests to qualify the purity of dNTP preparations are long distance PCR or the synthesis of long cDNAs in a reverse transcription reaction. dNTP quality is also a very important factor for real-time assays.

Q11. Is the pH of the dNTP solution important for stability?

Yes. The optimal pH for storage of nucleotides is from pH 7.5 –8.2 (pH at 20°C). During freezing / thawing cycles, the pH of the dNTP solutions can differ from the pH at 20°C.

The pH for Lithium salt solutions is not as temperature-dependent as with sodium salts, hence where Lithium salts are used, no dramatic shifts in pH occur when dNTPs are repeatedly frozen and thawed. This results in the dNTP preparation being more stable and, consequently, having a much longer shelf-life than with sodium salts.

Q12. Is dNTP concentration important for stability?

Yes. Significant hydrolysis can occur when dNTPs are stored at concentrations below 10mM. When storing nucleotide stock solutions for long periods, ensure that they are at a concentration well in excess of 10mM and preferably at 100mM.

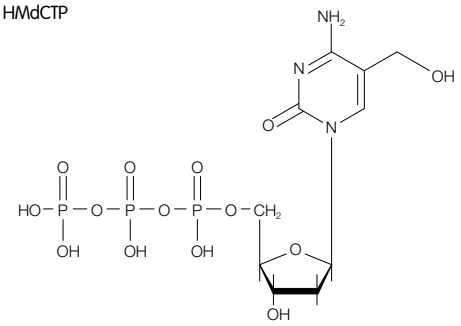
Q13. Is it important to have a dNTP preparation free from heavy and transition metals?

Yes. The presence of these metals increases degradation of dNTPs into dNDPs and dNMPs. Hence, metal-free preparations are more stable.

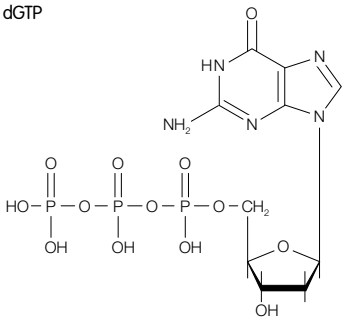
Q14. Which methods exist for manufacturing dNTPs?

dNTPs are normally manufactured from deoxynucleosides or deoxynucleoside monophosphates (dNMPs) by either chemical phosphorylation or enzymatic synthesis. Chemical synthesis involves the addition of phosphate groups or inorganic pyrophosphates (PPi) to deoxynucleosides or dNMPs, whereas the enzymatic method employed by Bioline involves a phosphorylation process carried out by highly specific enzymes.

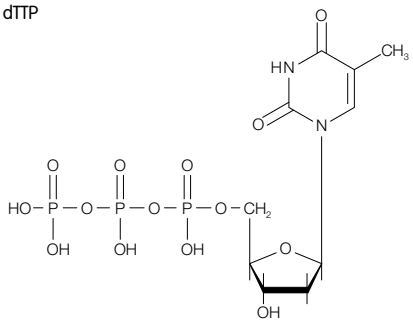
HMdCTP



dGTP



dTTP



Q15. What are the advantages of dNTP enzymatic synthesis over chemical synthesis?

The enzymatic synthesis of dNTPs uses highly specific enzymatic systems which eliminate impurities and PCR inhibitors, such as modified nucleotides, PPi and deoxynucleoside tetraphosphates. PCR reactions are impeded by the presence of contaminants resulting from chemical manufacturing processes, such as traces of dNDPs, pyrophosphates or other ionic species (e.g. acetate). Such contamination may lead to poor yields or to no PCR product at all. Unless thoroughly purified, chemically synthesised dNTPs often contain deoxynucleoside tetraphosphates which are powerful PCR inhibitors. Chemical synthesis can also lead to deamination and other nucleotide modifications whereas enzymatic synthesis of dNTPs bypasses these risks.

Enzymatic Phosphorylation cascade:

Q16. Are nucleotides in solution more stable than those in a lyophilised form?

Yes. Preparations of dNTPs decompose into nucleoside di- and mono-phosphates via a disproportionation reaction. At temperatures above 4°C, lyophilised preparations of deoxynucleotides undergo disproportionation faster than nucleotides in solution. By contrast, at -20°C, the rate of degradation for both forms is less than 1% per year. Nucleotides in solution are also generally purer. Some lyophilised preparations approach 98% purity or more but rarely match the >99% achieved with extremely pure solutions. Generally, nucleotides in solution are purer than the lyophilised version.

Q17. What are the advantages of dNTPs being presented in Lithium salts as opposed to in Sodium salts?

Reference has been made earlier to the greater solubility of dNTPs in Lithium salts than in Sodium salts. Also mentioned in question 11, dNTPs presented in Lithium salts are more resistant to repeated freeze/thawing than those presented in Sodium salts. Furthermore, they remain sterile during the entire storage period (the Lithium ion has been shown to have significant bacteriostatic activity towards various microorganisms).

Finally, using Lithium-salt nucleotide preparations reduces salt-induced artefacts and increases the legibility of sequencing gels. Lithium salts are highly suited to PCR sequencing and labelling applications.

Q18. Is it important to have a dNTP preparation free from inorganic pyrophosphate (PPi)?

Yes, an excess of inorganic pyrophosphate can inhibit PCR reactions since DNA replication is favoured by a low concentration of pyrophosphates on account of the hydrolytic action of cellular pyrophosphates. Inorganic pyrophosphate is often present in chemically synthesised dNTPs and this contamination can be detected by NMR detection and not by conventional HPLC methods. The enzymatic synthesis employed by Bioline produces dNTPS which are entirely free of inorganic pyrophosphate.

Q19. Is it important to have a dNTP preparation free from nucleoside tetraphosphates?

Yes, chemical dNTP synthesis is normally carried out by addition of PPi to dNMPs. If PPi is added to dNDP, it will result in the formation of nucleoside tetraphosphates which may case strand termination as manifested by gel “smearing” and other PCR problems. This is especially important in long-distance PCR applications and reverse transcription of long fragments. Such problems are not encountered with enzymatically synthesised dNTPs, since phosphorylation is carried out using highly specific enzymes that only incorporate one phosphate group at a time.

Validated Applications

Performance and Sensitivity

Bioline ultra-pure dNTPs guarantee outstanding results by undergoing functional tests with a wide range of assays. The stringent quality control includes tests for the absence of DNase, RNase, Protease, Nickase activity and absence of human, viral and bacterial DNA. Our dNTPs have been validated for use in a variety of molecular biology applications, including highly sensitive techniques such as long-range PCR, qPCR, RT-PCR, low-copy or rare-message assays.

Validated Applications

Standard PCR
Long-range PCR
qPCR
cDNA Synthesis / RT-PCR
Sequencing
Genotyping
Site-directed Mutagenesis
Microarrays
DHPLC

Exceptional PCR amplification

Impurities in PCR reagents can often inhibit PCR reactions. This is most often seen in long-range PCR methods.

Exceptional PCR Amplification

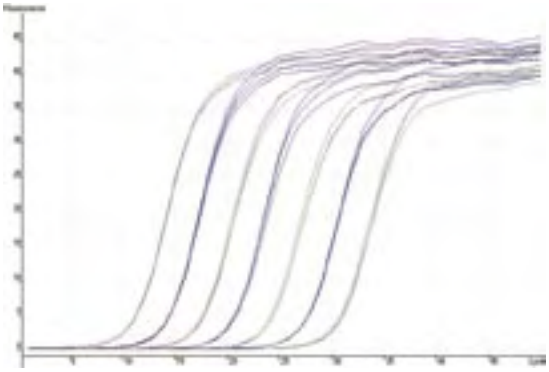
To demonstrate the high level of purity of Bioline dNTPs, a long-range 20Kb PCR reaction was performed using ultra-pure dNTPs and BIO-X-ACT Long DNA polymerase. Results were visualized by electrophoresis on a 1% agarose gel in 1x TAE stained with ethidium bromide.

Lane 1: Amplification of a 20Kb fragment of Lambda DNA
Lane 2: HyperLadder I

High Efficiency qPCR reactions

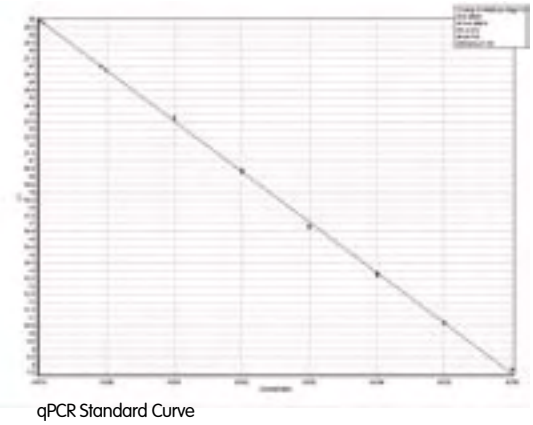
Real-time PCR is perhaps the most sensitive technique for gene expression analysis and is reliant upon the quality of reagents to yield reliable data. Bioline ultra-pure dNTPs used in combination with a superior hot-start DNA polymerase such as IMMOLASE™ are ideal for qPCR methods over a large range of cDNA / DNA template preparations. The figures below show results obtained in conjunction with IMMOLASE DNA Polymerase.

Outstanding results for qPCR



qPCR over a Broad Dynamic Range

Bioline dNTPs are validated for use in qPCR experiments. A fragment of the GAPDH gene was amplified and the results show exact replicates over a 1 x 10^7 dynamic range.



qPCR Standard Curve

Log DNA concentration is plotted against Ct. The data shows an efficiency of close to 1, indicating an exact doubling of product per cycle.

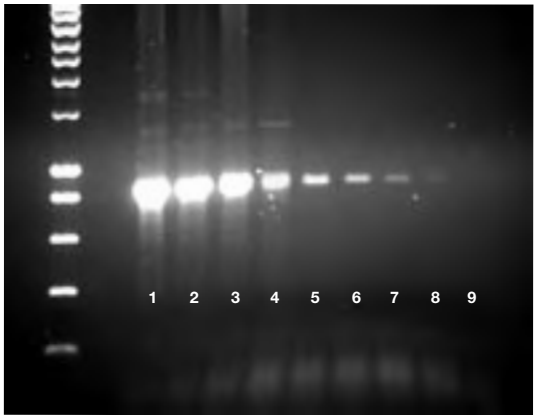
Generation of highest-quality cDNA

cDNA synthesis is the process by which RNA is used as a template and a complementary DNA strand is produced by a reverse transcriptase enzyme. Bioline ultra-pure dNTPs used in conjunction with BioScript™, (an ultra-stable Reverse Transcriptase) generate cDNA of the highest-quality, even with low amounts of template.

Low copy assays

Bioline ultra-pure dNTPs are validated for highly sensitive techniques to achieve outstanding results. The data below shows that extremely low quantities of template can be successfully amplified using ultra-pure NTPs in combination with BIOTAQ™ DNA polymerase.

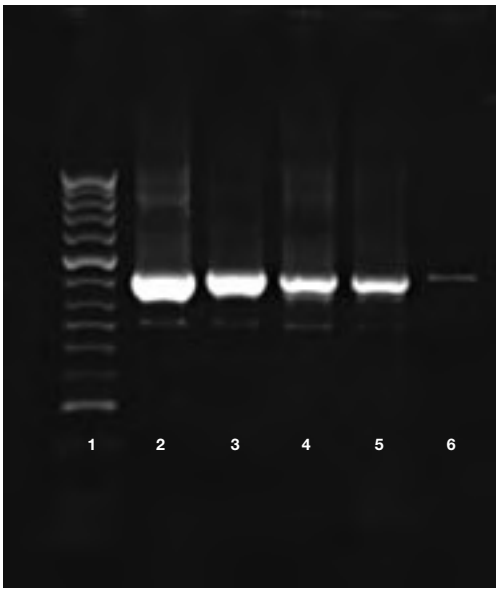
Generation of highest-quality cDNA



Reverse Transcription PCR

Template dilution experiment: Various quantities of total HeLa cell RNA were reverse-transcribed using Bioline dNTPs, BioScript and oligo(dT)$_{18}$ primer in a 20µl reaction, (1) 50ng, (2) 25ng, (3) 10ng, (4) 1ng, (5) 500pg, (6) 250pg, (7) 100pg, (8) 50pg and (9) 0pg. Subsequently, 5µl of each reaction was used in conjunction with a ß-Actin specific primer to amplify an 860bp band.

Outstanding results for Highly Sensitive Techniques



Low Copy Assay

A fragment of the ß-actin gene of human genomic DNA was amplified using Bioline dNTPs in a 50µl reaction volume, with different template concentrations.

Lane 1: HyperLadder II
Lane 2: 50ng of genomic DNA
Lane 3: 5ng of genomic DNA
Lane 4: 2.5ng of genomic DNA
Lane 5: 0.5ng of genomic DNA
Lane 6: 0.1ng of genomic DNA

DHPLC

DNA fragments amplified using Bioline dNTPs have been assayed for use on DHPLC. The purity of dNTP is a critical consideration for DHPLC usage, since there must be no contamination by monophosphates or incorrectly synthesised / modified bases. Contaminating nucleotides or derivatives may cause premature strand termination and result in unwanted peaks. Bioline dNTPs deliver specific melt profiles.

Ordering Information

Product Specification

	dATP	dCTP	dGTP
Product	dATP Lithium 100mM Solution	dCTP Lithium 100mM Solution	dGTP Lithium 100mM Solution
Nomenclature	2'-deoxyadenosine-5'-triphosphate	2'-deoxycytidine-5'-triphosphate	2'-deoxyguanosine-5'-triphosphate
Formula	$C_{10}H_{12}N_5O_{12}P_3Li_4$	$C_{10}H_{12}N_3O_{13}P_3Li_4$	$C_{10}H_{12}N_5O_{13}P_3Li_4$
Molecular Weight	514.916g/mol	490.891g/mol	530.916g/mol
λmax pH 7.0	259nm	272nm	252nm
ε at λmax	15.4 E x $mmol^{-1}$ x cm^{-1}	9.1 E x $mmol^{-1}$ x cm^{-1}	13.7 E x $mmol^{-1}$ x cm^{-1}
A_{250}/A_{260}	0.78 ± 0.03	0.82 ± 0.03	1.16 ± 0.05
A_{280}/A_{260}	0.15 ± 0.02	0.98 ± 0.03	0.66 ± 0.03
Concentration	100mM ± 2%	100mM ± 2%	100mM ± 2%
Appearance	clear colourless solution	clear colourless solution	clear colourless solution
pH of Solution	7.5	7.5	7.5
dNTP (HPLC Area)	≥99%	≥99%	≥99%
dNDP (HPLC Area)	<1%	<1%	<1%
DNases, RNases, Nicking Activity	Negative	Negative	Negative
Storage	at -20°C	at -20°C	at -20°C
Stability	≥24 months	≥24 months	≥24 months

Standard Product Range

Bioline ultra-pure dNTPs are available in a wide range of standard formats, as individual sets or as ready-to-use mixes. Our dNTP mixes can be added directly to amplification reactions, and are designed to save time, reduce the risk of contamination and ensure the reproducibility of results.

Custom, Bulk and OEM Nucleotide Service

When your requirements for dNTPs are beyond the scope of our standard product range, we invite you to take advantage of our custom, bulk and OEM nucleotide services. Bioline is a primary manufacturer of nucleotides and can accommodate requests for micro-litre to multi-litre quantities. We can manufacture special molecular biology grade nucleotide formulations, blends and mixes to your requirements. Private labelling and packaging arrangements are also available.

For bulk enquiries please contact info@dntps.com

Extended Storage Life

Bioline dNTPs have the distinct advantage of being presented as Li salts rather than as Na salts. dNTPs presented in Li salts are more resistant to repeated freeze / thaw cycles and remain sterile over the entire storage period (Lithium ions exhibit bacteriostatic activity towards most microorganisms). dNTPs are more soluble as Lithium salts than as Sodium salts. This is particularly important for dGTP which has a tendency to precipitate during freezing thereby causing an imbalance in the final dNTP concentration. Lithium salts are also more soluble in ethanol than Sodium salts, so their removal by ethanol precipitation is more efficient, as it reduces salt artefacts and increases the legibility of sequencing gels. Lithium salts are highly suited to PCR, sequencing and labelling applications.

Bioline dNTPs are stable for 24 months when stored in a –20°C constant-temperature freezer.

dTTP	dITP	dUTP	HMdCTP
dTTP Lithium 100mM Solution	dITP Lithium 100mM Solution	dUTP Lithium 100mM Solution	HMdCTP Lithium 100mM Solution
2'-deoxythymidine-5'-triphosphate	2'-deoxyinosine-5'-triphosphate	2'-deoxyuridine-5'-triphosphate	5-hydroxymethyl 2'-deoxycytidine-5'-triphosphate
$C_{10}H_{13}N_2O_{14}P_3Li_4$	$C_{10}H_{11}Li_4N_4O_{13}P_3$	$C_9H_{12}N_2O_{14}P_3Li_4$	$C_{10}H_{14}Li_4\ N_3O_{14}P_3$
505.903g/mol	515.9g/mol	492.884g/mol	520.9g/mol
267nm	249nm	262nm	275nm
9.6 E x $mmol^{-1}$ x cm^{-1}	12.2 E x $mmol^{-1}$ x cm^{-1}	10.0 E x $mmol^{-1}$ x cm^{-1}	7.7 E x $mmol^{-1}$ x cm^{-1}
0.65 ± 0.03	1.68 ± 0.04	0.75 ± 0.03	0.90 ± 0.03
0.73 ± 0.02	0.25 ± 0.02	0.38 ± 0.02	1.33 ± 0.03
100mM ± 2%	100mM ± 2%	100mM ± 2%	100mM ± 2%
clear colourless solution	clear colourless solution	clear colourless solution	clear colourless solution
7.5	7.5	7.5	7.5
≥99%	≥99%	≥99%	≥99%
<1%	< 1%	<1%	< 1%
Negative	Negative	Negative	Negative
at -20°C	at -20°C	at -20°C	at -20°C
≥24 months	≥24 months	≥24 months	≥24 months

dNTPs

DESCRIPTION	PACK SIZE	FINAL CONC.	CAT NO.
dNTP Set (dATP, dCTP, dGTP, dTTP)			
dNTP Set	4 x 25µmol	100mM Total	BIO-39025
dNTP Set	4 x 100µmol	100mM Total	BIO-39026
dNTP Set	4 x 500µmol	100mM Total	BIO-39027
dNTP Individual			
dATP	25µmol	100mM	BIO-39036
dCTP	25µmol	100mM	BIO-39038
dGTP	25µmol	100mM	BIO-39037
dTTP	25µmol	100mM	BIO-39039
dITP	25µmol	100mM	BIO-39032
dUTP	25µmol	100mM	BIO-39035
HMdCTP	25µmol	100mM	BIO-39046
dNTP Mix (dATP, dCTP, dGTP, dTTP)			
dNTP Mix	10µmol	10mM Total	BIO-39044
dNTP Mix	100µmol	10mM Total	BIO-39053
dNTP Mix	20µmol	40mM Total	BIO-39043
dNTP Mix	50µmol	100mM Total	BIO-39028
dNTP Mix	200µmol	100mM Total	BIO-39029

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