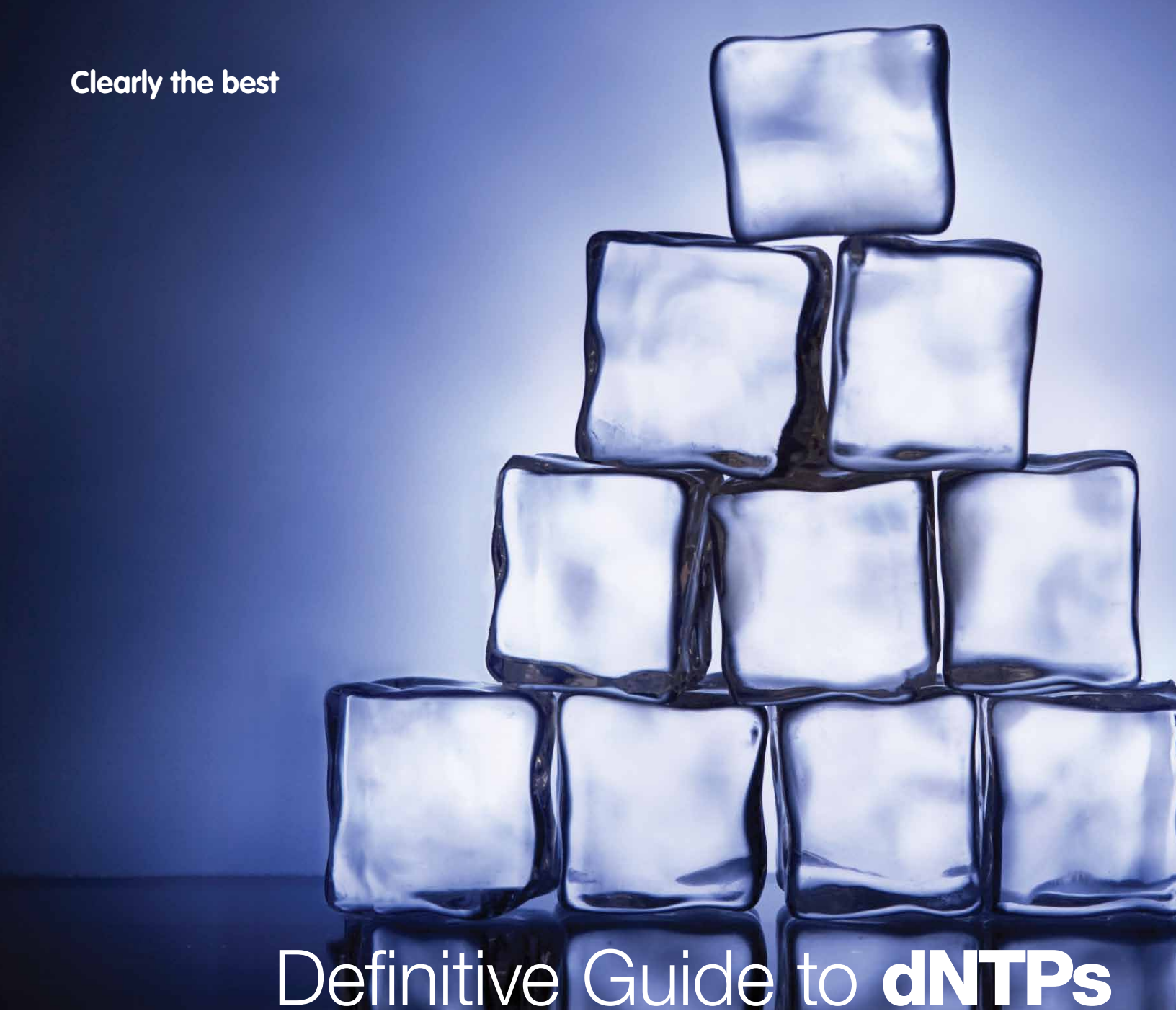
Clearly the best



Definitive Guide to **dNTPs**

Ultra-pure Nucleotides

BIOLINE

A Meridian Life Science® Company

Clearly the best

- >99% triphosphate purity by HPLC
- Free from PCR inhibitors
- DNase, RNase, Protease and Nickase free
- Supplied as individual dNTPs, sets and mixes
- Extended shelf-life of 24 months at -20°C
- Custom, bulk and OEM nucleotides

Contents

PRIMARY MANUFACTURER

Bioline, The PCR Company, is an international supplier of nucleotides, enzymes and other molecular biology tools for the life sciences field. From the company's foundation to the present, we have been actively involved in the development and manufacture of ultra-high purity deoxynucleoside triphosphates (dNTPs).

Bioline is one of only a few manufacturers of dNTPs in the world. Our ultra-pure dNTPs are synthesized under the international ISO 9001 standards for quality control.

ISO 9001:2008 Quality Management Certification Features:

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

Bioline's advanced nucleotide manufacturing facility in Germany

	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	$C_{10}H_{12}N_5O_{12}P_3Li_4$	$C_9H_{12}N_3O_{13}P_3Li_4$	$C_{10}H_{12}N_5O_{13}P_3Li_4$	
Molecular Weight	514.9g/mol	490.9g/mol	530.9g/mol	
Concentration	100mM ± 2%	100mM ± 2%	100mM ± 2%	
Appearance	Clear Colorless Solution	Clear Colorless Solution	Clear Colorless Solution	
pH of Solution	7.5	7.5	7.5	
Purity (HPLC)	≥99%	≥99%	≥99%	
DNase, RNase, Nicking Activity	Negative	Negative	Negative	
λmax pH 7.0	259nm	272nm	252nm	
ε at λmax @ pH7.0	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	
Storage	at -20°C	at -20°C	at -20°C	
Stability	≥24 months	≥24 months	≥24 months	

Stringent control systems guarantee that our dNTPs are of the highest quality and consistency to be found anywhere in the industry. A further assurance of this is the ISO certification of our purpose built state-of-the-art nucleotide manufacturing facilities in the Luckenwalde Biotechnology Park, Berlin-Brandenburg Life Science Cluster, Germany. With ISO certification implemented, Bioline demonstrates its commitment as a reliable and technically competent partner, for our customers, suppliers and future collaborators.

With increasing levels of sophistication of PCR processes and regulatory requirements, it has become essential to achieve highest dNTP quality, with minimal batch-to-batch variations. Bioline's ultra-high purity dNTPs are manufactured to the highest purity standards and are perfectly suited for a wide range of applications from PCR (including low copy, long-range, real-time), cDNA synthesis/reverse transcription assays, site-directed mutagenesis, DNA sequencing and microarrays.

	dTTP	dUTP	Hydroxymethyl dCTP
	dTTP lithium 100mM solution	dUTP lithium 100mM solution	Hydroxymethyl dCTP lithium 100mM solution
	2'-deoxythymidine-5'-triphosphate	2'-deoxyuridine-5'-triphosphate	5 hydroxymethyl 2'-deoxycytidine-5'-triphosphate
	$C_{10}H_{13}N_2O_{14}P_3Li_4$	$C_9H_{12}N_2O_{14}P_3Li_4$	$C_{11}H_{12}N_3O_{14}P_3Li_4$
	505.9g/mol	492.884g/mol	520.9g/mol
	100mM ± 2%	100mM ± 2%	100mM ± 2%
	Clear Colorless Solution	Clear Colorless Solution	Clear Colorless Solution
	7.5	7.5	7.5
	≥99%	≥99%	≥99%
	Negative	Negative	Negative
	267nm	262nm	275nm
	9.6 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	0.75 ± 0.03	0.90 ± 0.03
	0.73 ± 0.02	0.38 ± 0.02	1.33 ± 0.03
	at -20°C	at -20°C	at -20°C
	≥24 months	≥24 months	≥24 months

Purity of dNTPs is an essential starting parameter for any research application. PCR inhibitors and contaminants generally exert their effects through negative interaction with template, interference with DNA polymerases, or other means such as sequestration of essential co-factors. These can individually contribute to reducing the overall efficiency of polymerisation, causing the PCR to fail.

Bioline's ultra-pure dNTPs are manufactured to the highest purity standards in the industry. Our dNTPs are enzymatically synthesized from premium quality raw materials, using highly specific production systems in our purpose built facilities. The manufacturing process eliminates impurities and PCR-specific inhibitors such as modified nucleotides, tetraphosphates and pyrophosphates commonly observed in other commercially available dNTP products. Bioline's dNTPs undergo stringent purification steps including quantitative HPLC and possess ≥99% purity (fig. 1) to enhance incorporation and yield optimal results.

FUNCTIONAL PURITY BY RT-PCR

Reverse transcription PCR (RT-PCR) is one of the most sensitive assays for dNTP quality. When performed with limiting amounts of template, this allows for detection of even the smallest variations in dNTP performance (fig. 2).

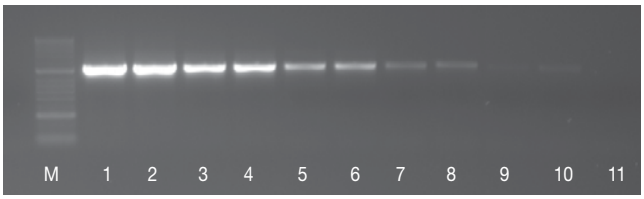


Fig. 2 Functional purity of Bioline's ultra-pure dNTPs in RT-PCR
Human total RNA was firstly reverse transcribed using Tetro reverse-transcriptase (BIO-65050). A five-fold serial dilution series of starting total RNA from 1µg to 1pg was performed. A 1kb fragment of the human *calnexin* gene was amplified using MyTaq™ HS in a 20µl reaction, incubated for 3 min at 95ºC followed by 35 cycles of 15s at 95ºC, 55ºC and 72ºC. Bioline's dNTPs yielded a visible product even down to 1pg of DNA template. Marker is HyperLadder™ II (M) (BIO-33054).

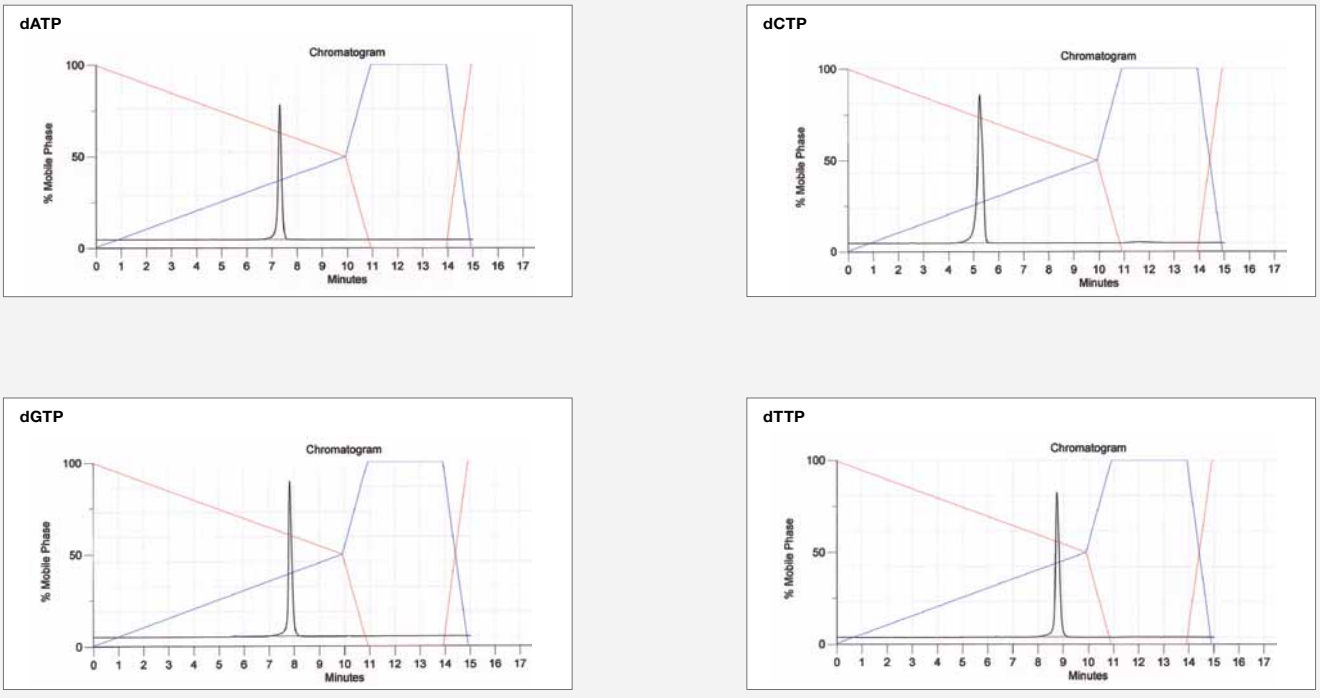


Fig.1 HPLC profile of ≥99% pure dNTPs
HPLC analysis shows greater than 99% triphosphate purity with undetectable mono-, di- and tetraphosphate forms.

OPTIMAL PERFORMANCE FOR REAL-TIME PCR

Bioline's dNTP mix is formulated for optimal performance in real-time PCR applications, which is the most sensitive technique for gene expression analysis, and is dependent upon high quality reagents to generate reliable data. Bioline's dNTPs are contained in the SensiFAST™ real-time product range, to help ensure that SensiFAST produces more consistent Ct values, especially at low input template concentrations (fig. 3).

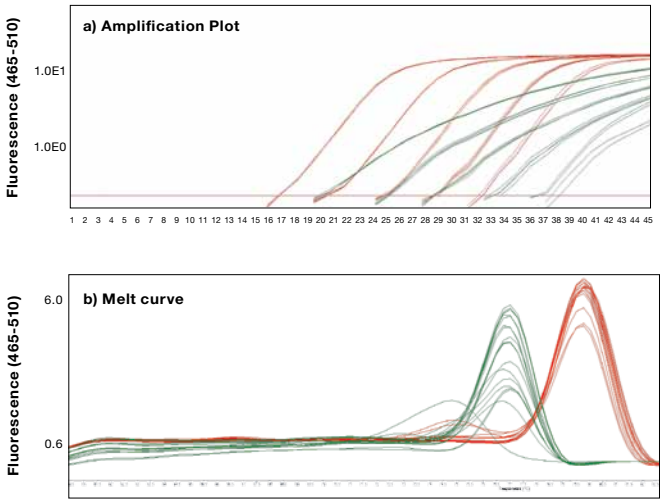


Fig. 3 Exceptional performance of Bioline's ultra-pure dNTPs in real-time PCR
The human *ß-actin* gene was amplified using SensiFAST™ SYBR Lo-ROX One-Step Kit (BIO-74001) (red) and the results compared with results from a One-Step Kit from supplier A (green). The experiment used ten-fold serial dilutions of human RNA (in triplicate) over 5 orders of magnitude. The conditions were 45°C for 10min followed by 95°C for 5min (15min for supplier A) and 35 cycles of 95°C 10s, 60°C 10s and 72°C 5s. The results illustrate that SensiFAST SYBR Lo-ROX One-Step is faster (earlier Ct values) with enhanced reproducibility (closer replicates) than supplier A.

EXCEPTIONALLY SENSITIVE RT-PCR

Bioline's ultra-pure dNTPs successfully generate cDNA of the highest quality and yield, even with very low template concentrations, making them ideal for highly sensitive reverse transcription (first-strand cDNA synthesis) and PCR (fig. 4).

SUCCESSFUL LOW COPY ASSAYS

Bioline's ultra-pure dNTPs are validated for highly sensitive techniques to achieve outstanding results. Figure 5 illustrates that extremely low quantities of template can be successfully amplified using Bioline's ultra-pure dNTPs.

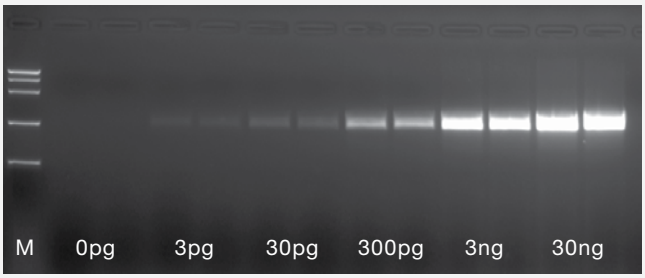


Fig. 4 Exceptional sensitivity in One-Step RT-PCR
A ten-fold serial dilution of mouse total RNA (30ng, 3ng, 300pg, 30pg and 3pg respectively) in duplicate was used with MyTaq™ One-Step RT-PCR Kit (BIO-65048). A 1kb fragment was produced using RN18S gene specific primer (incubated at 45°C for 40min) and PCR primers (95°C for 5min and 30 cycles at 95°C for 30s, 58°C for 30s and 72°C for 60s). The results illustrate that using Bioline's dNTPs, RT-PCR was successful even with very low template concentrations.

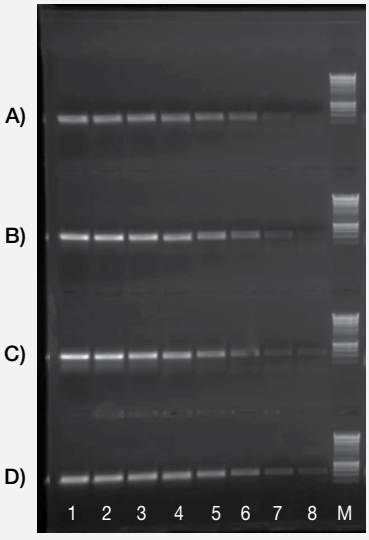


Fig. 5 Low copy assay of human genomic DNA
A) and B) a 340bp and 450bp fragment of the *myc* gene, respectively C) a 525bp fragment of the *EGFR* gene and D) a 530bp fragment of the *AGRI1* gene, were amplified using Bioline's dNTPs with MyTaq HS (BIO-21111) under fast cycling conditions. The PCR used a serial dilution of human genomic DNA (100ng, 33ng, 10ng, 4ng, 1ng, 33pg, 10pg and 3pg genomic DNA, lanes 1-8 respectively), incubated for 3 min at 95ºC followed by 35 cycles of 15s at 95ºC, 55ºC and 72ºC. Marker is HyperLadder I (M) (BIO-33025). This showed that extremely low amounts of DNA template can be successfully amplified using Bioline's ultra-pure dNTPs and MyTaq HS.

UNRIVALLED HIGH YIELD PCR EVEN WITH CHALLENGING TARGETS

Bioline's ultra-pure dNTPs are ideally suited for even the most demanding applications, such as PCR with high GC-rich DNA (fig. 6). Stable secondary structures in GC-rich templates not only cause resistance to melting, but cause DNA polymerases to stall, resulting in incomplete amplification products. Furthermore, GC-rich regions often contain secondary primer annealing sites from which spurious fragments are produced. GC-rich PCR commonly results in low yields of the target fragment, ladders of non-specific products, amplicons of the incorrect length, primer-dimers and/or complete reaction failure.

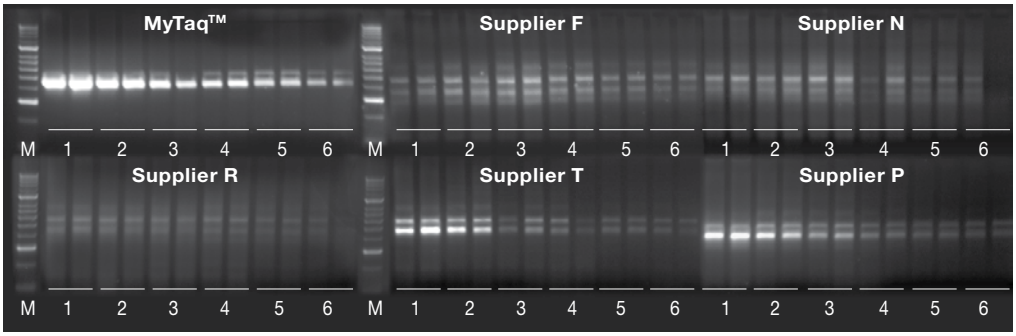
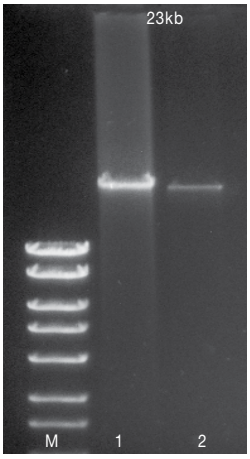


Fig. 6 Unrivalled high yield PCR with GC-rich human genomic DNA (61% GC content)
A GC-rich 450bp fragment of the human *myc* gene was amplified with Bioline's dNTPs and MyTaq™ (BIO-21105) and compared with DNA polymerase mixes and dNTPs from other suppliers. Decreasing amounts of human genomic DNA were used as a template in the PCR (1µg, 200ng, 100ng, 50ng, 25ng and 12.5ng; lanes 1-6 respectively). The cycling was performed under the following conditions: 95°C for 5 min, followed by 30 cycles at 95°C for 30s, 60°C for 30s and 72°C for 50s. Marker is HyperLadder I (M) (BIO-33025). The results illustrate that Bioline's dNTPs and MyTaq delivers much higher yield and sensitivity as compared with other suppliers.

HIGH PERFORMANCE LONG-RANGE PCR AMPLIFICATION

Impurities in PCR reagents can frequently inhibit reactions. For highly sensitive and accurate PCR techniques such as long-range PCR (over 20kb), high-purity nucleotides are particularly important for successful PCR reactions. Bioline's ultra-pure dNTPs have been functionally validated for high performance long-range PCR applications, ensuring highest amplification success rates (fig. 7).

A) Complex genomic DNA



B) λ DNA

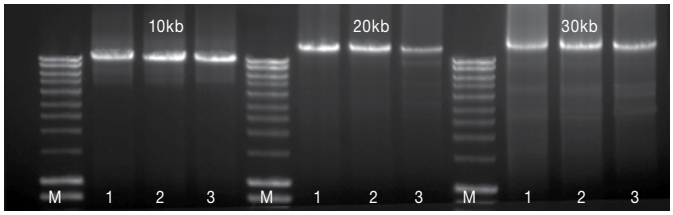


Fig. 7 Bioline's ultra-pure dNTPs are functionally validated for long-range PCR
A) A 23kb DNA fragment from the human γ-globulin gene was amplified with BIO-X-ACT™ short mix (BIO-25025). The PCR used human genomic DNA (25ng and 5ng, lanes 1 and 2 respectively). The cycling was performed under the following conditions: 95°C for 1 min, followed by 30 cycles at 98°C for 10s, 66°C for 18 min, and a final extension at 72°C for 20 min. PCR product was run on a 0.5% TAE agarose gel. Marker is HyperLadder I (M) (BIO-33025).
B) 10kb, 20kb and 30kb fragments of λ DNA were amplified with VELOCITY DNA polymerase (BIO-21098) using 50ng of λ DNA template (in triplicate). The cycling was performed under the following conditions: 98°C for 2 min, followed by 30 cycles at 98°C for 30s, 55°C for 30s, and 72°C for 5-15min (30s per 1kb). PCR product was run on a 0.8% TAE agarose gel. Marker is HyperLadder I (M), highest band is 10kb (BIO-33025). The results illustrate that Bioline's dNTPs are perfect for long-range PCR applications, using both simple (λ) and complex (genomic) DNA.

SUPERIOR dNTPS FOR THE MOST SENSITIVE MOLECULAR BIOLOGY APPLICATIONS

Bioline's ultra-pure dNTPs possess the highest level of purity in the industry and have been extensively validated for use in a wide variety of molecular biology applications.

Validated Applications		
Standard PCR	Hot-start PCR	Fluorescent DNA sequencing
Low copy PCR	Real-time PCR	Pyrosequencing
Long-range PCR (over 20kb)	cDNA synthesis/RT-PCR	Microarrays
Labelling reactions	Site-directed mutagenesis	Genotyping

EXTENDED STABILITY AND STORAGE

Bioline's ultra-pure dNTPs have the distinct advantage of being supplied as lithium salts in highly purified water at pH 7.5 to ensure functionality and long-term stability. Lithium salts have greater resistance to repeated freeze-thaw cycles than sodium salts (fig. 8A) and lithium salt dNTP preparations remain sterile over the entire shelf-life due to the bacteriostatic activity of lithium towards various microorganisms (fig. 8B).

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.

Our QC results demonstrate that Bioline's dNTP functionality is not only retained after multiple freeze-thaw cycles, but can also greatly exceed the standard 2 year storage recommendation time in a -20°C constant-temperature freezer.

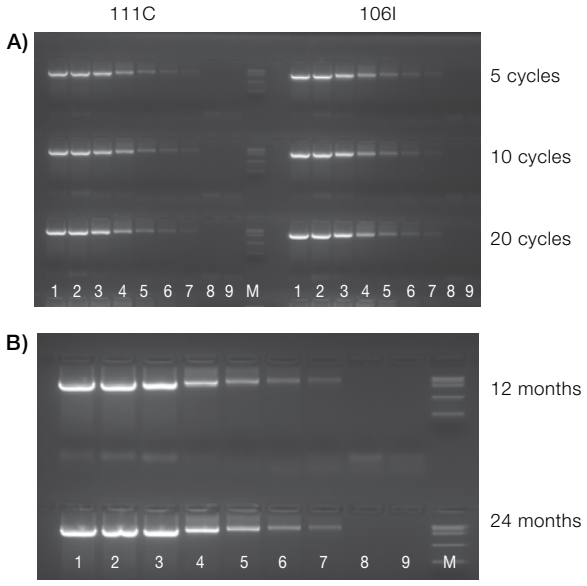


Fig. 8 Bioline's dNTP mix remains stable after multiple freeze-thaw cycles and long-term storage.
A) Bioline's 100mM dNTP mix (batch 111C and 106I) was freeze-thawed up to 20 times, and incubated with other PCR reaction components for 30 cycles of PCR. A two-fold serial dilution of Bioline's dNTP mix (100mM lane 1 to lane 9) was used with 10ng of λ DNA and amplified with BIOTAQ™ DNA polymerase (BIO-21040). Reactions with both batches produced identical PCR products of 2kb even if° freeze-thawed up to 20 times.
B) The stability of dNTPs was also assessed for functionality by storing at -20°C for up to 2 years. All dNTP batches performed exceptionally in PCR reactions with λ DNA (10ng), and amplified with BIOTAQ™ DNA polymerase regardless of age of the dNTPs.

BULK, OEM AND CUSTOM SERVICES

When your requirements are beyond the scope of our standard product range we invite you to take advantage of our proven world-class bulk, custom and OEM service. Our aim is to provide the best customer service and dNTP products in the industry.

Being a primary manufacturer of nucleotides, we can accommodate requests for micro-liter to multi-liter quantities. We can manufacture special nucleotide formulations, blends and mixes to your requirements. Private labelling and packaging arrangements are also available.

Manufactured under ISO 9001:2008 regulations, Bioline's ultra-pure dNTPs cost-effectively bring you the quality and supply security to meet your needs.

For bulk, custom and OEM service enquiries please contact: **custom@bioline.com**
For further information, please visit our dedicated microsite: **http://www.bioline.com/custom**

PRODUCTION FLEXIBILITY AT YOUR SERVICE

SCALE
- From micro-liters to multi-liters
- From mgs to kgs
- Special synthesis and batches

FORMAT
- Any concentration
- Any combination, formulation
- Any volume
- Custom mixes and blends

PRODUCTION PLANNING
- Batch reservation
- Lot retention samples
- Special testing as required
- Scheduled & global delivery

PACKAGING AND LABELING
- Any size
- Choice of tubes, vials & bottles
- Custom labeling
- Label design and printing
- Customized packaging
- OEM product finishing

SERVICE AND SUPPORT
- Validation support file
- Customer on-site audits
- Product traceability
- Confidentiality




Q&A

1. Why are dNTPs important?

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

2. How are Bioline's dNTPs supplied?

dNTPs can be supplied as either a mix or a set. 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 optimized for PCR and other applications. The set contains four separate tubes, one for each deoxynucleotide. dNTPs can be supplied in lyophilized form or in solution as either lithium or sodium salts.

3. 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 also offers 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. For MyTaq DNA Polymerase, the MyTaq buffer contains dNTPs.

4. 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 minimizing 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.

5. 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:

Nucleosidic Contaminants

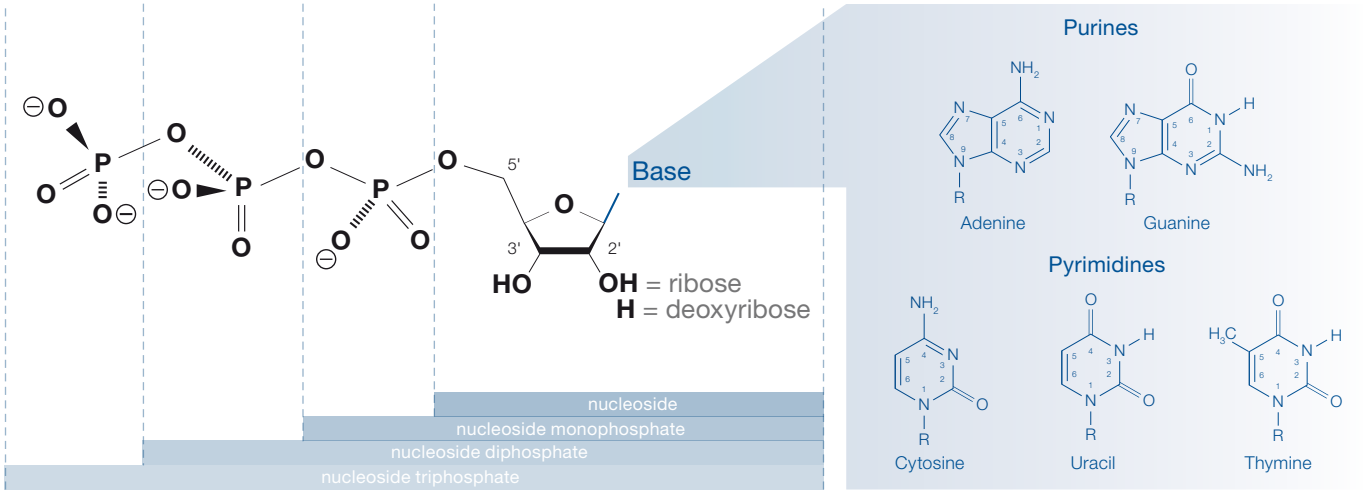
- Modified nucleotides such as nucleoside tetraphosphates may decrease the sensitivity or completely inhibit a PCR reaction
- Mono and diphosphate forms can decrease nucleotide incorporation
- Dideoxy base forms can terminate an amplification reaction

Macromolecular Contaminants

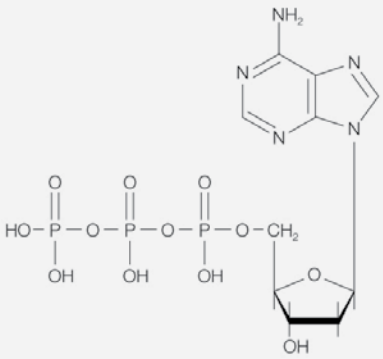
- DNAse and RNAse contaminants can affect cDNA synthesis resulting in false-negative results
- Nickase activity can compromise the amplification template
- Contaminating DNA from human and bacterial origins can result in false positives

Inorganic Species

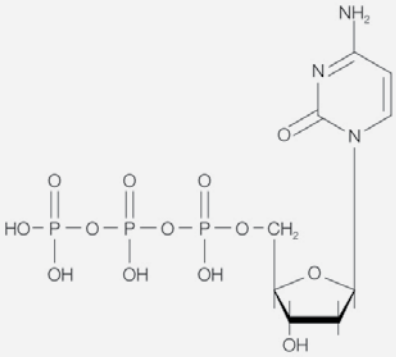
- Critical concentrations of inorganic species that interfere with PCR collectively termed as “PCR inhibitors” e.g. chloride, acetate, pyrophosphates, magnesium, calcium, total heavy metals *(Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, U)*
- Purity of nucleic acid templates is particularly important for real-time PCR as contaminants may interfere with fluorescence detection
- Incompatible buffer ions may lead to amplification of non-specific targets or may completely inhibit PCR reactions



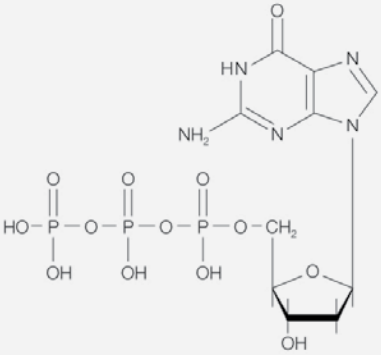
dATP



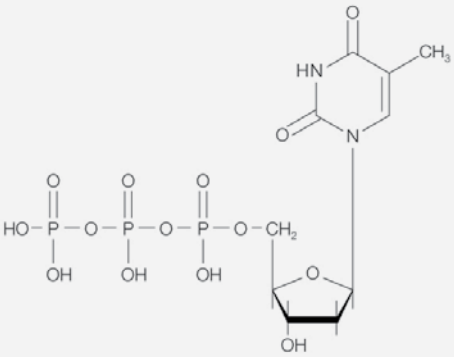
dCTP



dGTP



dTTP



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

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

7. Does dNTP quality affect fidelity?

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

8. Does dNTP quality affect processivity?

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

9. 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.

10. 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). An acidic pH will cause hydrolysis of dNTPs to dNDPs and dNMPs, rendering them less suitable for PCR applications. 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

11. 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.

12. 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.

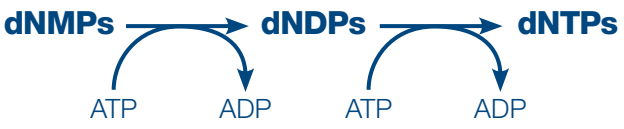
13. Which methods exist for manufacturing dNTPs?

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.

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

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 synthesized 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:



15. Are nucleotides in solution more stable than the lyophilized version?

Yes. Preparations of dNTPs decompose into nucleoside di- and mono-phosphates via a disproportionation reaction. At temperatures above 4°C, lyophilized 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 than the lyophilized form. Some lyophilized preparations approach 98% purity or more, but rarely match the >99% achieved with extremely pure solutions.

16. What are the advantages of dNTPs being presented in lithium salts as opposed to sodium salts?

Reference has been made earlier to the greater solubility of dNTPs in lithium salts than in sodium salts. Also, as mentioned in question 10, dNTPs presented in lithium salts are more resistant to repeated freeze-thaw cycles 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 micro-organisms). Finally, using lithium-salt nucleotide preparations reduces salt-induced artifacts and increases the legibility of sequencing gels. Lithium salts are highly suited to PCR sequencing and labeling applications.

17. 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 favored by a low concentration of pyrophosphates owing to the hydrolytic action of cellular pyrophosphates. Inorganic pyrophosphate is often present in chemically synthesized dNTPs and this contamination can be detected by NMR detection, not by conventional HPLC methods. The enzymatic synthesis employed by Bioline produces dNTPs which are entirely free of inorganic pyrophosphate.

18. Is it important to have a dNTP preparation free from 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 cause strand termination which manifest as 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 synthesized dNTPs, since phosphorylation is carried out using highly specific enzymes that only incorporate one phosphate group at a time.

19. Are the concentrations of your dNTP Mixes each or total?

All the concentrations of our dNTP Mixes are totalled, for example our 100mM dNTP Mix is made up of 25mM of each dNTP (dATP, dCTP, dGTP and dTTP).

20. I wish to prevent carry-over contamination of my PCR product using dUTP and UDGase, how does this work and which products are suitable?

When dUTP is used in place of, or in conjunction with dTTP, the resulting PCR product is a suitable substrate for Uracil DNA Glycosylase (UDGase), which allows the user to completely destroy any contaminating DNA from a previous PCR reaction prior to commencing the current amplification. At Bioline we provide dUTP both as a stand-alone product (BIO-39035) and as part of a dUTP Mix (BIO-39041).

21. I need to dilute my dNTPs to a different concentration, what should I use as a diluent?

We recommend that you dilute your dNTPs in PCR Grade water (BIO-37080).

Selected Publications

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Mayor, N.P., *et al. J. Immunol. Meth.* **327**(1/2), 82-87 (2007).
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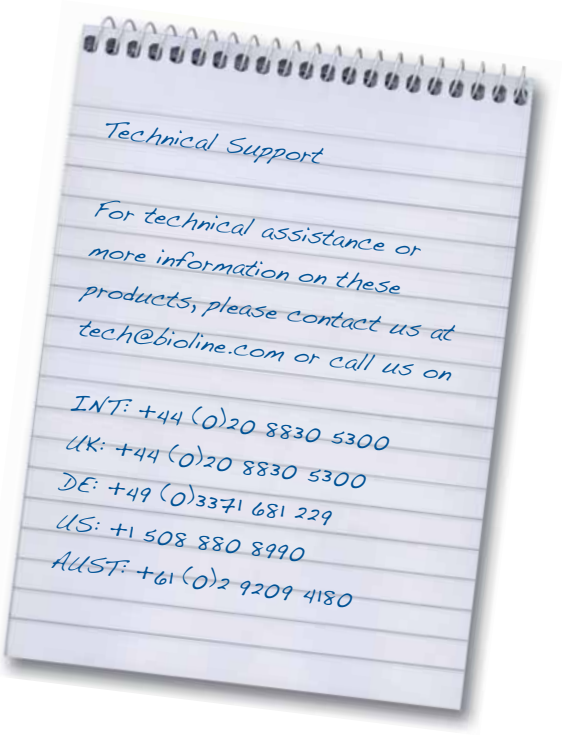
ORDERING INFORMATION

PRODUCT	CONC.	PACK SIZE	CAT NO.
dNTP Individual			
dATP	25µmol	100mM (1 x 250µl)	BIO-39036
dCTP	25µmol	100mM (1 x 250µl)	BIO-39038
dGTP	25µmol	100mM (1 x 250µl)	BIO-39037
dTTP	25µmol	100mM (1 x 250µl)	BIO-39039
dUTP	25µmol	100mM (1 x 250µl)	BIO-39035
HMdCTP	25µmol	100mM (1 x 250µl)	BIO-39046
dNTP Set (dATP, dCTP, dCTP, dTTP)			
dNTP Set	100mM total	4 x 25µmol (4 x 250µl)	BIO-39025
	100mM total	4 x 100µmol (4 x 1ml)	BIO-39049
	100mM total	4 x 100µmol (4 x 4 x 250µl)	BIO-39026
	100mM total	4 x 500µmol (4 x 20 x 250µl)	BIO-39027
	100mM total	4 x 1000µmol (4 x 10ml)	BIO-39055
dNTP Mix (dATP, dCTP, dGTP, dTTP)			
dNTP Mix	10mM total	10µmol (1ml)	BIO-39044
	40mM total	20µmol (1 x 500µl)	BIO-39043
	100mM total	50µmol (1 x 500µl)	BIO-39028
	10mM total	100µmol (10 x 1ml)	BIO-39053
	100mM total	200µmol (4 x 500µl)	BIO-39029
	40mM total	1000µmol (5 x 5ml)	BIO-39056
dUTP Mix (dATP, dCTP, dGTP, dUTP)			
dUTP Mix	50mM total	25µmol (1 x 500µl)	BIO-39041



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