Jena Bioscience

Building Blocks of Life

# dNTP Guide

## High Performance Nucleotides

- Primary manufacturer
- Custom formulations
- Micro to multi-litre solutions
- Exceptional performance

IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
Reg.-No.: ICV03597 034 and ICV03597 534

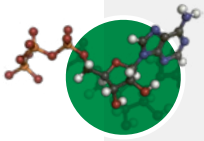
Molecular Biology

# Because nucleotides don’t get better than this.

- Ultrapure
- Exceptional performance
- Stable for 2 years at -20°C
- Custom Formulations
- OEM Supplier

Free from:

- Bacterial and Human DNA
- Potential Inhibitors
- DNases, RNases, Nicking enzymes
- Proteases



### Nucleotides & Nucleosides

In our chemistry division, we have hundreds of natural and modified nucleotides in stock. In addition, with our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from mg to kg scale.

### Click Chemistry, Probes & Epigenetics

Our Probes & Epigenetics as well as Click Chemistry sections offer innovative reagents for the functionalization, conjugation and labeling (fluorophores, haptens) of (bio) molecules complemented by epigenetic modification analysis tools.

### LEXSY Expression

In the field of recombinant protein production, Jena Bioscience has developed its proprietary LEXSY (Leishmania Expression System) technology. It is based on an S1-classified unicellular organism that combines easy handling with a eukaryotic protein folding and modification machinery. Besides everything you need to establish LEXSY in your lab we also offer custom expression of recombinant proteins.

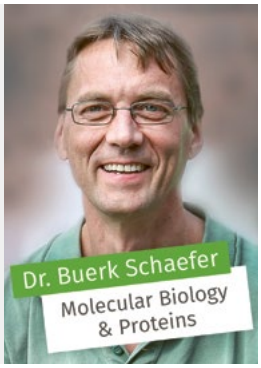
### Crystallography & Cryo-EM

For the crystallization of biological macro-molecules – which is often the bottleneck in determining the 3D-structure of proteins – we offer specialized reagents for protein stabilization, crystal screening, crystal optimization, and phasing that can reduce the time necessary to obtain a high resolution protein structure from several years to a few days.

### Molecular Biology & Proteins

For applications in the field of Molecular Biology we offer a large selection of single reagents, complete kits and optimized master mixes. This section includes products for DNA or RNA purification, amplification and modification with focus on PCR-related techniques.

For your questions regarding **dNTPs** contact me directly:
**pcr@jenabioscience.com**



# Jena Bioscience

Building Blocks of Life

Established in 1998 by a team of scientists from the Max-Planck-Institute of Molecular Physiology (Dortmund), Jena Bioscience utilizes more than 25 years of academic know-how to develop innovative reagents for clients from both research and industry in 100+ countries. To date, Jena Bioscience still remains an owner-operated business.

Jena Bioscience

**Imprint:**
Design & Layout by timespin - Digital Communication GmbH, Sophienstr. 1, D-07743 Jena, Germany, www.timespin.de | Copyright: Please contact Jena Bioscience if you want to use texts and/or images in any format or media.

For the sake of the environment: this brochure is printed on chlorine-free natural paper.

# Contents

Specifications ........ 6

Production Technology ........ 7

Quality ........ 8

- Purity 8
- Macromoleular Contaminants 8
- Inorganic Species 9

Supplier Comparison ........ 10

- Purity 10
- Functionality 11

Request a sample ........ 12

# Specifications

| | Test | Specification |
|---|---|---|
| **Physical** | Concentration[1] (22 °C, pH 7.0) | 100 – 110 mM |
| | Appearance | clear colorless solution |
| | pH (22 °C) | 8.5 ± 0.2 |
| | Stability (from certification date) | 24 months |
| **HPLC** | dNTP (C18-RP-UV) | ≥ 99.0 % (area) |
| | dNDP (C18-RP-UV) | ≤ 0.5 % (area) |
| | NTP or TMP for dTTP (C18-RP-UV) | ≤ 0.1 % (area) |
| **Functional** | Low Copy Long Range PCR (18 kb, lambda DNA, template dilution series) [2] | PCR fragment with 50 pg of template or less |
| | RT-PCR (749 bp fragment, human GAPDH gene, template dilution series) | PCR fragment with 10 pg of template or less |
| | Contamination with bacterial DNA (qPCR, 16S rRNA[3]) | not detectable |
| | Contamination with human DNA (qPCR, beta-actin gene[4]) | not detectable |
| | DNases, RNases, Nicking Activity (Gel electrophoresis) | not detectable |
| | Proteases (UV-Vis) | not detectable |
| **Anions & Cations** | Chloride $Cl^-$ (Anion chromatography) | ≤ 10 mM |
| | Acetate $CH_3COO^-$ (GC/FID) | ≤ 2 mM |
| | Magnesium $Mg^{2+}$ (ICP-MS) | ≤ 5 mM |
| | Calcium $Ca^{2+}$ (ICP-MS) | ≤ 0.25 mM |

[1] Cavaluzzi & Borer (2004) *Nucleic Acids Res.* **32(1)**:e13
[2] For dUTP: Low Copy PCR (1 kb, lambda DNA, template dilution series)
[3] Greisen *et al.* (1994) *J. Clin. Microbiol.* **32(2)**:335
[4] Fields *et al.* (2001) *Toxicol. Sci.* **63**:107
[5] Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, U

## dNTP Solutions

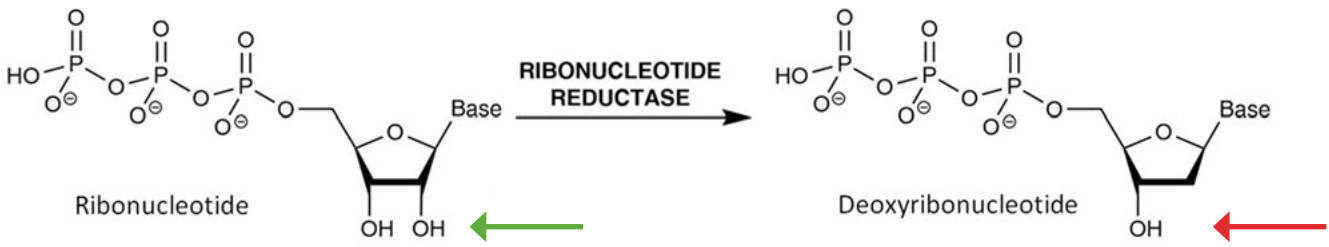
| | dATP sodium salt 100 mM solution | dCTP sodium salt 100 mM solution | dGTP sodium salt 100 mM solution | dTTP sodium salt 100 mM solution | dUTP sodium salt 100 mM solution |
|---|---|---|---|---|---|
| **Nomenclature** | 2'-Deoxyadenosine 5'-triphosphate | 2'-Deoxycytidine 5'-triphosphate | 2'-Deoxyguanosine 5'-triphosphate | 2'-Deoxythymidine 5'-triphosphate | 2'-Deoxyuridine 5'-triphosphate |
| **CAS No.** | 1927-31-7 | 102783-51-7 | 93919-41-6 | 18423-43-3 | 102814-08-4 |
| **Formula (free acid)** | $C_{10}H_{16}N_5O_{12}P_3$ | $C_9H_{16}N_3O_{13}P_3$ | $C_{10}H_{16}N_5O_{13}P_3$ | $C_{10}H_{17}N_2O_{14}P_3$ | $C_9H_{15}N_2O_{14}P_3$ |
| **Formula weight (g x mol$^{-1}$)** | 491.18 | 467.15 | 507.18 | 482.17 | 468.14 |
| **Molar Extinction Coefficient [1]** | ε = 15.1 l x mmol$^{-1}$ x cm$^{-1}$; 259 nm | ε = 8.9 l x mmol$^{-1}$ x cm$^{-1}$; 271 nm | ε = 14.2 l x mmol$^{-1}$ x cm$^{-1}$; 252 nm | ε = 9.5 l x mmol$^{-1}$ x cm$^{-1}$; 267 nm | ε = 9.8 l x mmol$^{-1}$ x cm$^{-1}$; 262 nm |

## Production Technology

Jena Bioscience is one of only a few primary manufacturers of dNTPs for PCR. Our high quality starts with our production technology. Many problematic impurities, such as pyrophosphate and modified nucleotides, are by-products from chemical synthesis. These impurities can severely impact PCR performance. That's why we synthesise all our dNTPs enzymatically, meaning many common impurities are never even present in our solutions.

Any remaining impurities are removed with several state-of-the-art purification procedures.

For dATP, dCTP, dGTP, and dUTP, we start with the respective ribonucleotide, and use the highly specific Ribonucleotide Reductase enzyme (Scheme 1). While for dTTP, we use enzymes to sequentially phosphorylate thymidine.




**Scheme 1.** *The bacterial enzyme ribonucleotide reductase selectively reduces the 2'-OH-group of the selected ribonucleotide (NTP) to give the corresponding Deoxyribonucleotide (dNTP). Our enzymatic synthesis is performed in this manner on a kilogram scale.*

# Quality

The level of sophistication in PCR applications constantly reaches new highs. Performance can be negatively affected by even one poor quality reagent. That's why we ensure the highest quality in our dNTP solutions. According to our ISO 9001:2015 certified quality management system each dNTP lot is assayed under stringent criteria for purity and functionality (see Specifications, page 6). The quality of a dNTP solution can be assessed with three criteria: Purity, Macromolecular Contaminants, and Inorganic Species.

## Purity

Purity of dNTPs is a deciding factor in PCR performance. A specification of ≥ 99.0 % dNTP is the market standard, yet this only tells you half the story. The constitution of the remaining ≤ 1.0 % is crucial.

Common contaminants in dNTP solutions are:

- Deoxyribonucleotide diphosphates – dNDPs
- Ribonucleotides – NTPs
- Other dNTPs – e.g. deaminated or methylated dNTPs
- Other nucleosidic compounds
- Phosphates

Of these contaminants, only the dNDPs minimally affect PCR performance. Even trace quantities of other impurities can significantly affect performance.

## Macromolecular Contaminants

The absence of human DNA and bacterial DNA in dNTP solutions is critical. These macromolecules can be present from the use of bacterial enzymes used in production, as well as human DNA from handling during production.

Since the presence of just a few copies of genomic DNA can cause false positives in PCR, we analyse each dNTP lot at the end of production to ensure they are DNA-free. (Figure 1) In addition, we test for residual enzymatic activity to ensure absence of DNases, RNases, Nicking activities, as well as proteases.

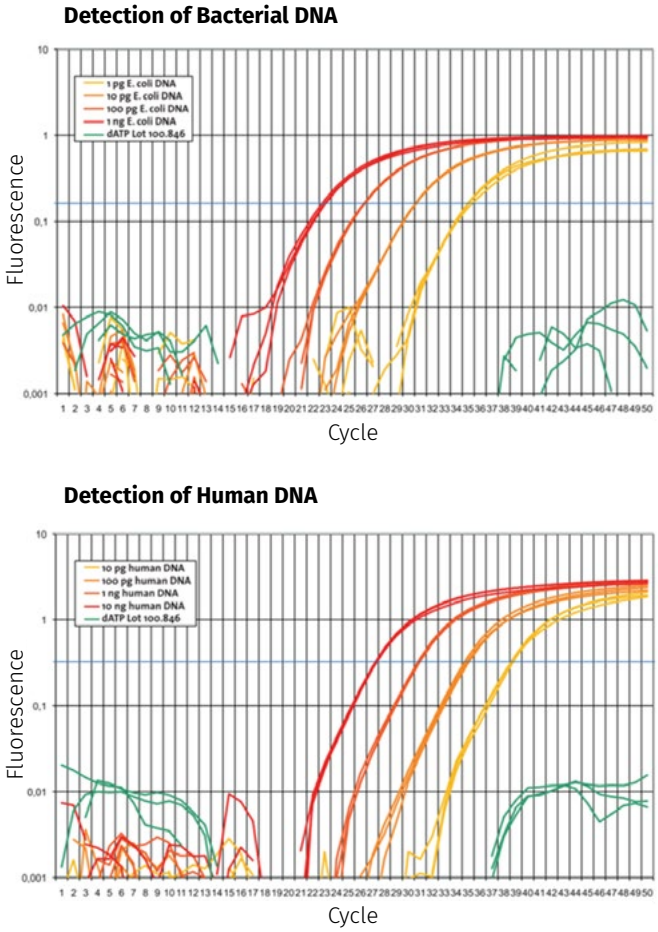



**Figure 1.** *A multiplex qPCR assay verifies the absence of contaminating bacterial or human DNA in Jena Bioscience dNTP solutions. The 16S rRNA gene is amplified for detection of bacterial DNA. Human DNA is detected by amplification of a beta-actin gene fragment. Traces of contaminating DNA are typically detected at ct-values between 35 and 45.*

## Inorganic Species

The presence of critical concentrations of inorganic species may result from contaminated raw materials and inadequate manufacturing processes. These species commonly interfere with PCR and are commonly referred to as “PCR inhibitors”. These do not absorb UV light and are not detected by UV-detection in reverse phase HPLC. To ensure our products are free from inhibiting quantites of these species, we use Analytical Anion Chromatography, GC/FID, and ICP-MS for analyses.

| **Species** | **Concentration reported to be critical in PCR** | **Specification for Jena Bioscience dNTPs** | |
|---|---|---|---|
| | | **in PCR mixture (0.2 mM = dilution 1:500)** | **in 100 mM dNTP solutions** |
| **Chloride $Cl^-$** | 25 mM | ≤ 0.02 mM | ≤ 10 mM |
| **Acetate $CH_3COO^-$** | 5 mM | ≤ 0.004 mM | ≤ 2 mM |
| **Magnesium $Mg^{2+}$** | 1.5 mM [1] | ≤ 0.01 mM | ≤ 5 mM |
| **Calcium $Ca^{2+}$** | 1 mM | ≤ 0.0005 mM | ≤ 0.25 mM |

[1] Standard concentration in PCR
[2] Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, U

# Supplier Comparison

To ensure our position as market leaders in quality, we routinely carry out extensive comparisons with other dNTP suppliers. These tests cover both purity and functionality.

## Purity

To accurately assess purity, we have optimised HPLC methodology to allow for separation of similar components. Figure 2 shows a example comparison of Jena Bioscience dATP solution to four dATP solutions from other suppliers. The peaks for dATP and dADP are labelled; other peaks present are potentially harmful to PCR performance.

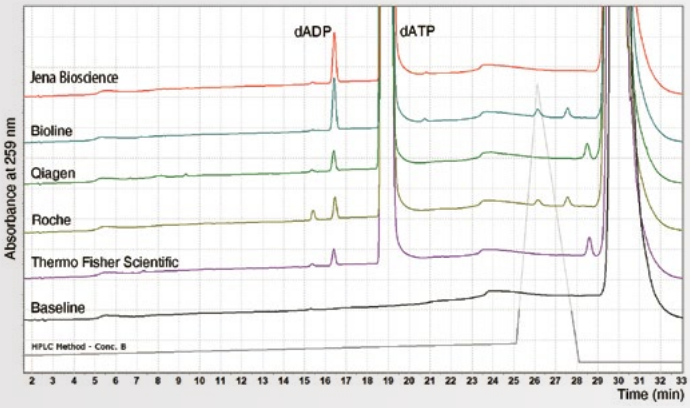



**Figure 2** *Comparison of HPLC traces for Jena Bioscience dATP solution with four other leading suppliers. Jena Bioscience dATP contains fewer impurities – such as nucleosidic compounds which can inhibit PCR enzymes.*

## Functionality

Functionality and performance in PCR assays is the most important factor for dNTP solutions. The four dNTPs (dATP, dCTP, dGTP, and dTTP) from each supplier were used to create a dNTP mix (25 mM each). These mixes were first tested in an 18 kb PCR reaction - amplification of such a large DNA fragment requires exceptional performance and can be negatively affected by impurities in dNTP solutions (Figures 3).

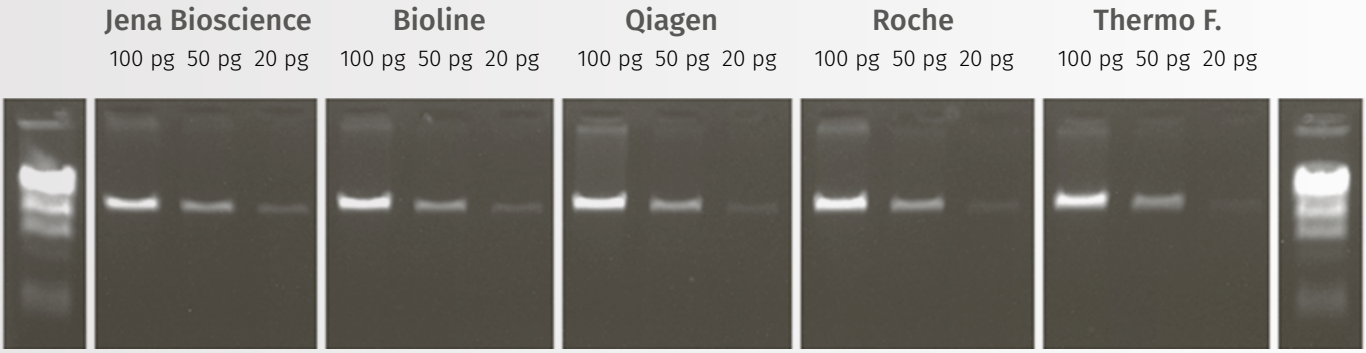



**Figure 3** *The individual dNTP solutions from each supplier were used to create a dNTP mix (25 mM each). Each mix was tested in an 18 kb PCR amplification with template dilution (left to right: 100 pg, 50 pg, 20 pg).*

Nucleotide impurities, such as NTPs, and modified dNTPs can strongly inhibit PCR enzymes. Therefore we use an assay particular sensitive to nucleotide impurities to evaluate all our dNTP solutions. The comparison of the dNTP mixes in this assay are shown in Figure 4.

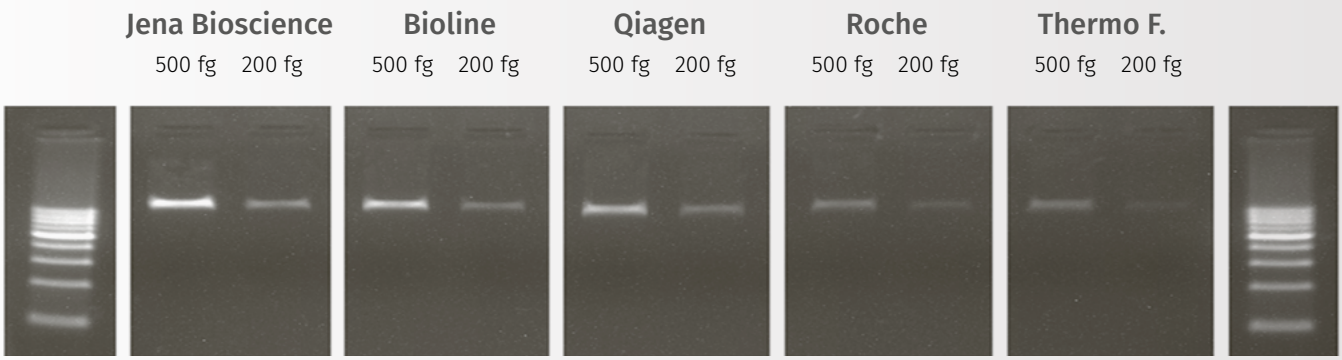



**Figure 4** *Each mix was used to amplify a 5 kb fragment in an assay particularly sensitive to nucleotide impurities. Lambda DNA was used as template (left to right: 500 fg, 200 fg)*

## Summary

- All suppliers have a dNTP purity over 99.5 %
- Jena Bioscience has a higher quantity of dNDP – essentially harmless to PCR
- Jena Bioscience has a significantly lower quantity of other impurities – those reducing PCR performance
- Jena Bioscience dNTP solutions gave clear bands in the 18 kb amplification.
- Jena Bioscience dNTP solutions performed exceptionally well in the assay sensitive to nucleotide impurities.

Jena Bioscience
Building Blocks of Life

Jena Bioscience GmbH
Loebstedter Str. 71
07749 Jena
Germany

Phone +49 (0)3641- 62 85 000
info@jenabioscience.com

www.jenabioscience.com

Test the exceptional performance of our nucleotides for yourself.

# Request a sample!

Our entire product range is available as samples.
We can provide multiple production lots of the following:

- Individual dNTP solutions (100 mM)
- dNTP mixes (25 mM each or 10 mM each)
- NTP solutions (100 mM)
- Custom formulations
- Lyophilisates

IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
Reg.-No.: ICV03597 034 and ICV03597 534

Molecular Biology